SPATIAL AND TEMPORAL VARIATION IN FEATHER CORTICOSTERONE IN HOUSE SPARROWS (*PASSER DOMESTICUS*) AND EURASIAN TREE SPARROWS (*PASSER MONTANUS*)

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Abstract

We currently lack knowledge of how the hormone corticosterone (CORT) varies across broad spatial and temporal scales. In birds, CORT acts to make energy available for routine tasks and is also secreted in response to stressors. Evaluating CORT physiology is a powerful tool for determining how individuals are coping with their environment. While CORT has traditionally been measured instantaneously, from plasma, a novel technique for measuring CORT deposited into feathers during growth (CORTf) provides a more holistic, integrated measure of hormone secretion over days to weeks. In addition to exploring how CORT varies in space and time, the present study shows that CORTf is associated with weather variables, corroborating previous findings obtained using plasma CORT.

House sparrow (*Passer domesticus*) CORTf was high in individuals sampled in the hot, dry, north-central region of Mexico. In this population, CORTf was also negatively related to measures of temperature and precipitation. Weather conditions outside of the moult period were stronger predictors of CORTf than conditions experienced during feather growth, indicating that past energetic challenges may have lasting effects on CORT physiology.

Over 27 years, variation in CORTf of Eurasian tree sparrows (*Passer montanus*) in Illinois was not linear, and showed a notable increase in the early 1980s. Tree sparrow CORTf was consistently negatively related to temperature. However, as CORTf was positively associated with spring precipitation and negatively associated with late summer precipitation, temperature-precipitation interactions appear to influence overall energetic requirements.

This is the first macrophysiological study using CORTf and these results provide valuable data that can be used as a reference point in future CORTf studies. These findings bring us closer to understanding what habitat conditions are energetically challenging and conversely what conditions are ideal for bird populations. This information is critical for identifying causes

of population declines and developing effective conservation measures. The ability to retrospectively analyze CORT using feathers is a highly innovative approach, and by understanding past responses to variation we can more accurately predict how future environmental change will impact populations.

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List of Abbreviations

ACTH	adrenocorticotrophic hormone
AIC	Akaike's information criterion
AVT	arginine vasotocin
BBS	breeding bird survey
CORT	corticosterone
CORTf	feather corticosterone
CORTfe	fecal corticosterone
CORTp	plasma corticosterone
CRF	corticotrophin-releasing factor
CV	coefficient of variation
ENSO	El Niño Southern Oscillation
GC	glucocorticoid
GLM	general linear model
HPA	hypothalamo-pituitary adrenal
NCDC	National Climatic Data Center
NMDS	non-metric multidimensional scaling
PBS	phosphate-buffered saline
PCA	principal components analysis
PDSI	Palmer drought severity index
ROM	Royal Ontario Museum

Chapter 1: Literature Review

1.1 Introduction to ecophysiology

Ecophysiology is the study of the interplay between the physiology and ecology of organisms living in natural environments (Bradshaw 2003). While the close association between an organism's environment and physiological processes such as thermoregulation has long been recognized, ecophysiology did not develop as a discipline until the 1950s (Block and Vannier 1994). By integrating physiology and ecology we can gain a new perspective on basic questions such as why a species is found in some areas but not others and why processes such as reproduction, growth, and recruitment vary spatially. Ecophysiological studies can also provide relevant information for conservation and management as understanding how individual physiological responses vary within and between populations can shed light on species' abilities to adapt to changing environmental conditions. Indeed, a recent paper identifying five 'grand challenges' for future research in organismal biology stated: "Advances in the area of organismal–environmental research will require interdisciplinary thinking and collaboration…understanding the flexibility and responses of physiological systems to climatic change is also critically important." (Schwenk *et al*. 2009).

1.2 Stress and corticosterone

Stress is widely recognized as an ecological factor that is critically important for the fitness and well-being of wild animals (Broom 1993; Newton 1998; Bonier *et al*. 2009). Thus, stress has emerged as a common focus of ecophysiological studies. Defining "stress" is difficult, as the term is used variably to refer to three different, although related, aspects of stress physiology: an unpredictable, unpleasant stimulus that an animal is exposed to; the physiological and behavioural responses of the animal to this stimulus; and the negative consequences that result from over-stimulation of these responses (Romero 2004). In an attempt to alleviate this

confusion, the reactive scope model was developed (Romero *et al.* 2009). This graphical representation of the effects of physiological responses to stressors within four distinct ranges is highly useful as it focuses on how stressors affect an individual. 'Predictive Homeostasis' refers to the range of levels of the physiological mediator involved in responding to predictable events such as breeding, migration, or seasonal changes in weather. 'Reactive Homeostasis' describes the range of levels of the mediator required to respond to stressors, defined as unpredictable events that threaten fitness. These two ranges comprise the 'normal reactive scope' of an individual, and when levels of the physiological mediator fall either above or below the normal reactive scope (ranges termed 'Homeostatic Overload' and 'Homeostatic Failure', respectively), pathology may develop.

The reactive scope model can be applied to any physiological mediator involved in responding to stressors. Corticosterone (CORT) is frequently chosen as the hormone of interest in studies focusing on the ecophysiology of stress. CORT is the main glucocorticoid (GC) hormone found in birds, reptiles, and rodents and it is produced via activation of the hypothalamo-pituitary-adrenal (HPA) axis. The process begins when an animal experiences a stimulus, and in response neural impulses are sent to the hypothalamus via the central, peripheral, and autonomic nervous systems (Toates *et al.* 1995). This causes the hypothalamus to synthesize and secrete corticotropin-releasing factor (CRF), arginine vasotocin (AVT), and oxytocin or mesotocin, which travel to the anterior pituitary (Wingfield and Ramenofsky, in Balm, Ed. 1999). At the pituitary these hormones stimulate the release of adrenocorticotrophic hormone (ACTH), which acts to promote the synthesis and release of GC hormones by the adrenal cortex. While the HPA axis is the most common route via which CORT is released into

the bloodstream, in some cases 'systemic stressors' are thought to activate the pituitary directly, without the involvement of the nervous system (Toates 1995).

CORT is well-studied because it is involved in or can affect three important processes: energy regulation, responding to stressors, and immune function. GC hormones mobilize stored amino acids, promote glucose synthesis (gluconeogenesis) in the liver, and stimulate the breakdown of stored fats (lipolysis), actions which help regulate energy expenditure (Karasov and Martínez del Rio 2007). Importantly, these effects indicate that CORT secretion is not always tied to responding to a specific stressor, and an individual's level of circulating CORT may simply reflect their current level of overall energetic demand. This line of reasoning is supported by a study showing that in many species plasma CORT (CORTp) levels fluctuate predictably on annual time scales, with CORTp levels being highest during breeding, a highly energetically demanding period (Romero 2002). However, this study also found that in several species of passerine birds, CORTp levels reach an annual low during moult and migration, also energetically costly stages. This indicates that there are likely multiple variables affecting plasma GC levels.

The most frequently cited stimulus causing GC secretion is exposure to stressors. When an animal experiences an unpredictable, noxious stimulus (a stressor) CORT is secreted and acts to redirect energy toward essential activities. CORT secretion allows the organism to respond to the stressor appropriately then return to normal (i.e. maintain homeostasis) when the stressor has passed (Wingfield *et al*. 1998). Examples of this redirection of energy are suppression of reproductive behaviour, promotion of foraging, and promotion of irruptive or escape behaviour (Wingfield and Ramenofsky 1999). This interruption of normal activities has been referred to as the 'emergency life-history stage', and is adaptive in that it increases an animal's chances of

surviving the stressor and enhances their ability to recover in a timely manner. However, it is important to note that if an animal experiences frequent or prolonged stressors, or if their ability to shut off CORT secretion via negative feedback and return to normal once the stressor has passed is impaired, elevated CORT levels can have serious, detrimental consequences (Sapolsky *et al.* 2000; Romero *et al.* 2011). The negative effects of chronic, elevated CORT levels can include suppression of growth, death of neuronal cells, breakdown of skeletal muscle, and suppression of the immune system (Wingfield and Ramenofsky 1999).

The discovery that GCs can inhibit the immune system initially led to doubts as to whether changes in these hormones could be considered an adaptive response to stressors (Maule and VanderKooi in Balm, Ed. 1999). The relationship between CORT and immunity is complex. Short-term CORT elevation can act to enhance immune activity, and the type of stressor an animal experiences can also influence how CORT affects immune function (Martin 2009). Additionally, CORT affects some facets of the immune system more strongly than others, and the specific immune function impacted can depend on the time-scale of exposure to elevated CORT (Martin 2009). A study that exposed neonatal rats to a toxin also showed that early life exposure to immune challenges, and potentially to other stressors as well, can lead to increased sensitivity of the immune system to GC effects later in life (Shanks *et al*. 2000). There is also evidence that the sensitivity of the immune system to GC effects can vary between populations, as temperate house sparrows (*Passer domesticus*) showed a reduction in immune activity in response to an artificial CORT increase but tropical house sparrows showed no change in immune function (Martin *et al*. 2005).

1.3 Broad-scale variation in corticosterone and relationships between corticosterone and weather variables

By understanding how physiological responses vary both within and between populations on broad scales we can identify potential drivers of this variation and make predictions regarding the level of plasticity and adaptation that may be seen in these traits in response to future changes. While there is considerable interest in exploring broad-scale variation in physiological traits and also in increasing our understanding of stress physiology, these research topics are seldom combined. In terms of spatial variation, several studies have compared CORTp levels between high altitude and low altitude populations or between populations from northern and southern areas within a species' range (ex. Silverin and Wingfield 1998; Bears *et al*. 2003). The majority of these studies were designed to test the 'short season' hypothesis, which posits that birds at high latitudes or altitudes have a limited time period in which they can breed, face frequent environmental perturbations, and are often unable to re-nest; therefore these birds should down-regulate the HPA axis so that CORT increases do not interfere with breeding activities. As predicted based on this hypothesis, most studies have found negative relationships between CORTp and both latitude and elevation (see Wingfield *et al*. 1994, Silverin *et al*. 1997, Silverin and Wingfield 1998, O'Reilly and Wingfield 2001, Meddle *et al*. 2003, Wilson and Holberton 2004). However, some studies found positive CORTp-latitude relationships (Wingfield *et al*. 1995, Martin *et al*. 2005), no relationship (Lynn *et al*. 2003), or an association that changed between years (Lindström *et al*. 2005). Clearly more work is needed to resolve these conflicting results and to expand our knowledge to include stages other than the breeding period.

These studies focused on CORT levels during breeding. We lack information about CORT-latitude and CORT-altitude relationships during other stages of the annual cycle.

Another caveat is that these studies only compared birds from two sites chosen to represent 'extremes' in terms of latitude or altitude so we do not have a comprehensive picture of CORT variation across an entire species' range. A recent study addressed this knowledge gap by measuring CORTp levels of song wrens (*Cyphorhinus phaeocephalus*) along a 45 km transect that covers the majority of the species' range on the isthmus of Panama (Busch *et al*. 2011). CORTp levels were higher and body condition was lower in birds living in dry areas near the species' range limit. This may indicate that this species cannot expand its range into drier regions because individuals are unable to physiologically cope with these energetically demanding conditions.

Broad-scale temporal variation in CORT is another question that has not been properly addressed, as the vast majority of CORT studies take place over a period of <5 years. To date, just one study has explored long-term CORT variability. Bortolotti *et al*. (2009a) compared feather CORT (CORTf) levels of great horned owls (*Bubo virginianus*) collected in 2004-2005 to CORTf levels of museum specimens collected from 1931-1974 and found that CORTf levels were higher in the museum specimens. This could indicate that historical conditions were more energetically demanding or could reflect a sampling bias related to the health status of individuals at the time of capture. The potential to expand our knowledge of long-term variability in GC levels has recently been made possible by the advent of techniques for measuring CORT from feathers (Bortolotti *et al*. 2008) and CORT or cortisol from hair (Sauvé *et al*. 2007). As these methods require only dead tissue they can be applied to museum specimens, opening up a multitude of possibilities for studying long-term changes in GC levels within populations and using this information to predict responses to future environmental change.

Despite our lack of knowledge regarding long-term CORT variation, on short time scales there is often considerable inter-annual variation in CORT. A two-year study of effects of food supplementation on CORT in Florida scrub jays (*Aphelocoma coeruloscens*) found that across all treatments CORTp was lower in the second study year, which was unusually favourable as indicated by increased adult body condition and high fledging rates (Schoech *et al*. 2007). In individual common murres (*Uria aalge*), CORTp levels were higher in a prey mismatch year compared to a match year, suggesting that inter-annual differences in food availability can drive temporal patterns in CORT variation (Doody *et al*. 2008). Kittiwake (*Rissa tridactyla*) chicks showed higher stress-induced CORTp levels in low productivity years which were characterized by poor foraging conditions but this relationship was not found in baseline CORTp levels (Brewer *et al*. 2008). In contrast Beletsky *et al*. (1992) found that mean annual CORTp levels of red-winged blackbirds (*Agelaius phoeniceus*) did not differ significantly among 4 study years. This may indicate that inter-annual differences may only occur when 'extreme' years, such as a prey mismatch year, are included in the study period.

Many of the aforementioned studies have linked inter-annual differences in CORT levels to variation in food availability. Weather is another important environmental factor that varies between years, and precipitation can affect the abundance of food resources, indirectly contributing to CORT variation. It is important to clearly distinguish weather, short-term events or states taking place over hours to days, from climate, which refers to average weather conditions over a period of several years (Wingfield and Ramenofsky 2011). While weather conditions are pervasive in the lives of wild animals, two recent studies highlighted our lack of understanding of how weather affects basic patterns and processes such as population dynamics (Knape and de Valpine 2011) and species distributions (Zuckerberg *et al*. 2011). Furthermore,

we would expect CORT to be responsive to weather conditions because GC hormones are involved in energy regulation and weather can influence daily energetic requirements directly, for example by altering the costs of thermoregulation, or indirectly via effects on food availability. Extreme weather events such as storms or droughts could also be perceived as stressors, which would be reflected in CORT levels.

Many studies have focused on the effects of storms or periods of extreme, inclement weather, on CORT levels. Diving petrels (*Pelecanoides urinatrix*) captured during a period characterized by high winds, low temperatures, snow, and decreased visibility had higher CORTp levels than birds captured before the storm (Smith *et al*. 1994). Similarly Lapland longspurs (*Calcarius lapponicus*) captured after a 3-day snowstorm had higher acute CORTp levels compared to birds captured before the storm (Astheimer *et al*. 1995). Snowfall was also associated with increased CORTp levels in dark-eyed juncos (*Junco hyemalis hyemalis*; Rogers *et al*. 1993) and in subordinate Harris' sparrows (*Zonotrichia querula*; Rohwer and Wingfield 1981). In white-ruffed manakins (*Corapipo altera*), CORTp was positively associated with periods of extreme precipitation (Boyle *et al*. 2010). Cold, rainy periods during breeding were associated with higher CORTp levels in song sparrows (*Melospiza melodia*) and white-crowned sparrows (*Zonotrichia leucophrys pugetensis*; Wingfield *et al*. 1983; Wingfield 1985a,b). However, these effects differed between the sexes and also depended on whether the storm occurred during an early or late stage of the breeding period. Wind can also affect CORT, as cliff swallows sampled during an extremely windy period showed elevated CORTp levels compared to those sampled before and after the windy period (Raouf *et al*. 2006).

CORT increases in response to extreme, inclement weather can be explained in the context of the emergency life-history stage as they allow the animal to respond adaptively to

these perturbations or stressors (Wingfield *et al*. 1998). Recent studies have begun exploring the effects of more moderate weather changes on GC levels. Negative relationships have been found between CORTp and fecal CORT (CORTfe) and mean, minimum, or maximum temperatures (Jenni-Eiermann *et al*. 2008; Frigerio *et al*. 2004; Lobato *et al*. 2008). Interestingly greylag geese (*Anser anser*) also showed a positive relationship between CORTfe and minimum afternoon air pressure the day before sampling, which may indicate that they use environmental cues to physiologically "prepare" for future, weather-based changes in energetic requirements (Frigerio *et al*. 2004). A lab study tested the CORT-temperature association by exposing starlings to a moderate (3 °C), experimental decrease in temperature and found that cooled birds had higher post-treatment CORTp levels than controls (de Bruijn and Romero 2011). Precipitation can also influence CORT secretion. Dusky flycatchers (*Empidonax oberholseri*) had higher baseline CORTp in years with longer periods of precipitation prior to sampling (Pereyra and Wingfield 2003), and nestling alpine swifts (*Apus melba*) sampled following cool, rainy mornings with high winds had elevated baseline CORTp compared to nestlings sampled after warm, dry, calm mornings (Bize *et al*. 2010). The effect of precipitation on CORT is likely indirect, as rain and snow are known to have negative effects on foraging success (Wingfield and Ramenofsky 2011) and lab studies have shown that CORTp levels increase in response to reduced or unpredictable food availability (Pravosudov *et al*. 2001; Reneerkens *et al*. 2002).

Cyclical weather events, such as the El Niño Southern Oscillation (ENSO), may also influence CORT levels as these conditions affect resource availability. However studies thus far have yielded mixed results. Some species showed increased CORTp in El Niño years (Wingfield and Ramenofsky 2011) and others showed no clear relationship between CORTp and ENSO phase (Addison *et al*. 2008; Wingfield and Ramenofsky 2011). These discrepancies

highlight the difficulty in drawing firm conclusions about relationships between CORT and weather conditions. These associations can vary between species and also based on sex (Wingfield 1985a,b), age class (Schwabl *et al*. 1985), and social status (Rohwer and Wingfield 1981; Rubenstein 2007). Romero *et al*. (2000) also found that within a species the sensitivity of the HPA axis to weather can vary between life-history stages, as three arctic-breeding passerines showed weak or non-existent relationships between CORTp and breeding season weather but strong correlations between CORTp and weather during the moult period. The pronounced effects of weather on CORT levels during moult are interesting. However, many of the previously mentioned studies have focused on the breeding period and examination of CORT-weather relationships during other life-history stages is warranted.

1.4 Plasma, fecal, and feather corticosterone measurements

Corticosterone levels of wild or captive animals can be measured in several ways. The most prevalent technique used is blood sampling. Because this procedure is a stressor in itself, a capture and restraint protocol is used to obtain two samples representing CORTp levels before and after the stressor is experienced (Romero and Wingfield 2001). In the field, a blood sample is taken within three minutes of capturing the animal to obtain the "baseline" CORTp level then the animal is held in a bag or other enclosure for approximately 30 minutes after which a second blood sample is taken to obtain the "acute" or "stress-induced" CORTp level. Theoretically, the baseline sample reflects the level of CORT circulating in the blood prior to the disturbance of capture, and the acute sample represents the increased CORT level present in the blood after the HPA axis has been activated by the investigator-induced stressor of capture, handling, and sampling.

There are several limitations to the blood sampling method. Baseline blood samples can be difficult to obtain in the field. It may be impractical to constantly monitor traps to determine the exact moment of capture and to extract animals from mist nets or other traps within the 3 minute window. There is also evidence that in some cases CORTp levels may begin to increase as soon as 1.5-2 minutes following capture (Schoech *et al*. 1999, Romero and Reed 2005). This means that samples collected >90 seconds post-capture may not accurately represent baseline CORTp levels. There is also the question of whether baseline samples truly represent an "unstressed" CORTp concentration. As blood sampling yields an instantaneous measure of CORT physiology it is impossible to know whether the baseline level may have been elevated, for example by an encounter with a predator or a conspecific prior to capture, unless the animal was tracked before it was sampled. Another concern is that acute CORTp levels reflect CORT secretion occurring in response to the stressor of capture and handling by the investigator, and it is difficult to determine how this response may be related to CORT responses to natural stressors of varying duration and magnitude. Finally, while some studies have concluded that blood sampling does not negatively affect wild birds (ex. Sheldon *et al*. 2008), blood sampling resulted in a 21-33% decrease in average survivorship in cliff swallows (Brown and Brown 2009). In light of this result, using blood sampling to measure CORT raises concerns in terms of animal welfare and investigator-induced effects on individual survival. These effects would be especially problematic for studies focusing on endangered or threatened species or studies examining links between CORT and fitness or survival.

In response to these concerns, several non-invasive techniques for measuring CORT have been developed. Measuring CORT from feces (Harper and Austad 2000; Goymann *et al*. 2002) or cloacal fluid (Hiebert *et al*. 2000) have the advantages of being non-invasive, providing

measures of hormone secretion integrated over a period of hours, and theoretically avoiding bias due to investigator-induced CORT responses. However, CORTfe levels can be influenced by diet or by individual variation in how hormones are processed by the kidney and liver (Creel 2001). Cloacal fluid CORT levels must be corrected to account for the hydration state of the individual being sampled (Hiebert *et al*. 2000). In baboons (*Papio cynocephalus cynocephalus*) variation in the amount of dietary fiber affects excretion of progestogens in feces (Wasser *et al*. 1993), and variation in dietary fiber content may also affect the measurement of glucocorticoid metabolites from feces (Goymann *et al*. 2005). This issue can be alleviated to an extent by expressing hormone concentrations as grams per dry weight to control for the amount of water in feces but CORTfe measurements still tend to be "noisier" than plasma measures (Creel 2001). It has also been shown that in some species sex differences in glucocorticoid metabolism may prevent comparative analyses (Goymann 2005). Finally, while these methods readily lend themselves to lab situations, obtaining samples of feces or cloacal fluid in the field can be challenging and in some cases impossible.

Relatively new, non-invasive techniques for measuring CORT from hair (Macbeth *et al*. 2010) and feathers (Bortolotti *et al*. 2008) are appealing to many ecophysiologists because the logistics of sampling and storing these tissues in the field are much simpler than those involved in sampling blood or excreta. Additionally, blood sampling provides an instantaneous measure of CORT secretion but CORTf is an integrated, long-term measure reflecting circulating plasma CORT concentrations during the days to weeks over which the feather is grown (Bortolotti *et al*. 2008). This method is also ideal for controlled studies evaluating treatments effects because feather growth can be induced outside of the moult period by pulling a feather. Daily growth of the induced feather can then be measured, and the feather can be cut into 'pre-treatment' and

'post-treatment' sections which can be compared to assess how circulating CORT levels changed in response to the manipulation. As previously mentioned this technique also opens up new possibilities for retrospective exploration of variation in CORTf levels using museum specimens, as it appears that CORT in feathers does not degrade over time (Bortolotti *et al*. 2009a).

Clearly there are many advantages to the feather CORT technique. The method was independently validated by implanting starlings (*Sturnus vulgaris*) with CORT and showing that the increased plasma hormone concentrations were reflected in CORTf values (Lattin *et al*. 2011). Studies linking CORTf to a variety of ecological variables have also yielded notable results. In red-legged partridges (*Alectoris rufa*) CORTf was related to clutch size in females and to feather colouration in males, which acts as a social signal in this species (Bortolotti *et al*. 2008). Experimental manipulations have also shown that CORTf influences how red grouse (*Lagopus lagopus scoticus*) respond to parasite and testosterone treatments (Bortolotti *et al*. 2009b) and how parasite load affects development of ornaments (Mougeot *et al*. 2010). Environmental conditions can also influence CORTf. Nestling male tree swallows (*Tachycineta bicolor*) from a reclaimed wetland site had higher feather CORT than nestlings from a reference site (Harms *et al*. 2010) and in Dupont's larks (*Chersophilus duponti*) CORT was associated with habitat conditions as indexed by stable isotope (δC13) ratios (Fairhurst 2011). On a smaller scale, nest box type (traditional plywood boxes versus thicker-walled aspen boxes) was associated with CORTf of nestling tree swallows, while adult females showed relationships between CORTf and laying date, clutch size, and productivity (Fairhurst *et al*. 2012a). In captive Clark's nutcrackers (*Nucifraga columbiana*) the addition and removal of environmental enrichment were both associated with CORTf increases but interestingly responses were related to the time-scale of exposure to enrichment objects (Fairhurst *et al*. 2011). Finally, CORTf has

been shown to predict future survival of wild house sparrows (Koren *et al*. 2012), indicating that this measure can be used as a biological indicator or biomarker.

1.5 Objectives, hypotheses, and predictions

Two main objectives of my project are to explore spatial variation in CORTf in house sparrows across their range in Mexico and to investigate temporal variation in CORTf in Eurasian tree sparrows (*Passer montanus*; hereafter "tree sparrows") in Illinois from 1963-1990. My final objective was to evaluate relationships between CORTf and weather variables, focusing on temperature and precipitation, in each of these populations. This work provides information that can be used for comparison with new data (i.e. "baseline" data) and potentially for predicting future patterns in CORTf as environmental conditions change. I also use my results as a basis for generating new hypotheses that can be experimentally tested in the future.

This project aims to fill a gap in our understanding of how CORT varies over broad-scales in both space and time. Information about spatial variation can help determine whether populations may be able to cope with future environmental change through changes in distribution, plasticity of the CORT response, or both. Retrospective CORTf data will provide a baseline that can be used to assess whether current and future CORTf fluctuations fall within the normal range of variation (i.e. the normal reactive scope, Romero *et al*. 2009) for a given species, or alternately if these fluctuations are likely to negatively impact individual condition and ultimately populations. Understanding factors that contribute to habitat suitability is critical for effectively conserving bird populations. This project can make a major contribution toward developing this knowledge by demonstrating that CORTf is a highly useful measure for assessing habitat quality, or the energetic costs or benefits of certain habitat features. Finally, this study will be the first to explore CORTf-weather relationships. This is important for

addressing gaps in our understanding of how wild animals perceive and cope with weather conditions. Also, this analysis will determine whether CORTf will show associations with weather similar to those found in studies that used CORTp or CORTfe.

Though I did not test pre-established hypotheses, I had several predictions regarding the CORTf relationships that I expected to emerge from each data set. In house sparrows I predicted that CORTf would be highest in individuals living in low rainfall areas in Mexico, in keeping with the findings of Busch *et al*. (2011). I also predicted that CORTf would be negatively related to both altitude and latitude in this population. This prediction may seem counter-intuitive, as in temperate regions high-altitude or high-latitude areas are considered to be more severe or demanding environments and therefore we would likely expect non-breeding sparrows to show higher CORTf in these areas. However, house sparrows evolved in temperate areas and have colonized tropical regions relatively recently. Due to this evolutionary history I hypothesize that tropical areas may be more challenging for this species. Warm, humid conditions are farther from their physiologically optimal conditions than high-latitude or high-altitude regions of Mexico, which more closely resemble environments in which they evolved. Additionally, in low-altitude, low-latitude areas house sparrows may be exposed to novel parasites, predators, or competitors, all of which could increase energetic demands and therefore CORT levels.

My final prediction for house sparrows was that CORTf would be negatively related to ambient temperatures during the moult period. I based this prediction on the concept of the thermoneutral zone of an endotherm, which is the range of temperatures in which the organism does not have to expend large amounts of energy to maintain its body temperature. This zone is bounded by the upper and the lower critical temperatures, and for house sparrows from various

locations in the U. S. the lower critical temperature has been designated as 20-22 °C and the upper critical temperature as 37-38 °C (Hudson and Kimzey 1966). In Mexico, house sparrows will likely encounter temperatures low enough to cause increased energetic demands, and possibly also high temperatures that exceed the upper limit of their thermoneutral zone. Therefore, an alternate prediction was that the relationship between CORTf and temperature will be complex, with the lowest CORT values being associated with intermediate temperatures (i.e. temperatures that fall within the thermoneutral zone for house sparrows).

In tree sparrows, I predicted that there would be a gradual increase in CORTf over time. This prediction was based on Breeding Bird Survey data which shows that the Illinois population of tree sparrows has increased in size from 1966-2010 (estimated yearly percent change of +6.9%; Sauer *et al*. 2011) while their range has not expanded considerably over this time period (Barlow and Leckie 2000). In terms of relationships with weather I predicted that CORTf would be negatively related to temperature during the moult period, a prediction that was again based on the thermoneutral zone concept. It is unlikely that temperatures in Illinois will reach highs that exceed the upper limit of the thermoneutral zone of tree sparrows (reported as 36.4°C; Deng and Zhang 1990) even during the moult period (late summer – early fall). However, the lower critical temperature for this species has been reported as 27.5°C (Deng and Zhang 1990), and while this seems high even if the actual lower bound of the thermoneutral zone is closer to the value reported for house sparrows (20-22 °C; Hudson and Kimzey 1966) it is likely that these birds will experience challenging, low temperatures. These cold periods would be characterized by increased energetic demands and therefore increased CORT levels are expected. I also predicted that CORTf would be negatively related to precipitation. I based this prediction on the assumption that dry conditions will be more energetically challenging for tree sparrows, largely

due to presumed positive effects of precipitation on food availability. However, an alternate prediction was that CORTf will show a more complex relationship with total precipitation. Low CORT values could be found at intermediate levels of precipitation and higher CORT values found at low levels of precipitation due to the theorized positive association between precipitation and food availability, and also at high levels of precipitation due to increased energetic demands, for example because heavy rainfall can impair the foraging abilities of small birds (Keller and van Noordwijk 1994; Radford *et al*. 2001).

CORTf is an exciting physiological measure because it provides a window onto individual energetic state. It is essential to learn more about how CORTf is related to a range of ecological conditions to develop its potential to fill research gaps in the fields of ecophysiology and conservation. Obtaining information about how CORTf varies in space and in time is an essential first step to understanding which environmental factors have pronounced energetic consequences for birds. As weather is a constant factor in the lives of wild animals, it is also important to expand our knowledge of individuals' abilities to cope with fluctuations in temperature and precipitation. The objectives of this project also advance our understanding of how CORTf can be most effectively used by conservation biologists as a tool for assessing which habitat features are essential to maintain healthy populations.

Chapter 2: Spatial variation in feather corticosterone and CORT-weather relationships in House Sparrows (*Passer domesticus*) in Mexico

2.1 Introduction

Combining ecology and physiology has led to considerable advancement in these fields, and the advantages of using data collected at broad scales to answer biological questions has long been recognized, however more work is needed to unite these concepts. Macrophysiology was introduced in response to a growing interest in exploring physiological patterns on broad spatial and temporal scales (Chown *et al*. 2004). Macrophysiology seeks to study variation in physiological traits at large scales and to use this information to generate novel, holistic answers to basic questions in ecophysiology. By expanding the range of physiological studies we can also use current patterns to predict future responses to climate or land use changes (Chown and Gaston 2008). Since the introduction of macrophysiology this approach has been applied to a variety of contexts and taxa. For example, recent studies have investigated factors controlling patterns of species abundance and biological invasions in marine systems (reviewed in Osovitz and Hofmann 2007), predicted which tropical insect species are most vulnerable to climate change effects (Bonebrake and Deutsch 2012), and explored relationships between physiological traits and range size in beetles (Calosi *et al*. 2010). Despite these advances there are still many areas of physiology that have yet to be explored from a broad-scale perspective. One example is the study of variation in hormone levels within and between populations and how these patterns are related to environmental factors.

Corticosterone, the main avian glucocorticoid, is often referred to as a stress hormone, yet it is correlated with a multitude of ecological variables that do not all involve a stress response. CORT is associated with stress because it is produced and secreted when an animal experiences an unpredictable environmental perturbation or stressor (Wingfield *et al*. 1998; Romero 2004).

However, CORT is also involved in glucose synthesis and in the breakdown of fat, processes that provide energy for routine tasks such as foraging and social interactions (Dallman *et al*. 1993; Toates 1995). Studies have shown that individual CORTp and CORTfe levels are related to predation risk, social status, population density, and weather conditions (ex. Berger *et al*. 2007; Creel 2001; Lindstrom *et al*. 2005; Nephew and Romero 2003; Selva *et al*. 2011; Romero *et al*. 2000; Bize *et al*. 2010).

While most ecophysiological studies use blood sampling to measure CORT, feather CORT has several advantages over this technique. Blood sampling involves taking a 'baseline' measure within 3 minutes of capture and an 'acute' measure approximately 30 minutes after capture (Schoech *et al*. 1999; Romero and Romero 2002). Blood measures provide instantaneous pictures of individual CORT levels, which can be useful for inferring responses to manipulations or relating hormone levels to conditions experienced immediately prior to sampling. However, downsides to measuring CORT from blood include this restricted temporal perspective, the difficulty of obtaining baseline samples in the field, and potential negative effects on survival resulting from the invasive sampling procedure (Romero and Reed 2005; Brown and Brown 2009). Measuring CORT levels from feathers provides a longer-term perspective on CORT secretion and integrates both baseline levels and any elevations occurring during the period of feather growth (Bortolotti *et al*. 2008). Feather CORT also allows investigators to avoid the difficulty and negative effects of blood sampling. Furthermore, as initial work indicates that CORT in feathers does not degrade over time this technique opens up the possibility of using museum specimens to study long-term changes in a physiological measure (Bortolotti *et al*. 2009a). Initial work showed that CORTf is correlated with ecological variables including clutch size, social signals, and habitat conditions (Bortolotti *et al*. 2008;

Harms *et al*. 2010; Fairhurst 2011); and found an association between CORTf and future survival probability in house sparrows (Koren *et al*. 2012). Clearly this measure provides a powerful tool for expanding our knowledge of ecophysiological relationships in a variety of contexts.

Though Busch *et al*. (2011) explored variation in CORTp of song wrens across the Isthmus of Panama, feather CORT has not yet been used to address macrophysiological questions. The objective of this study is to explore variation in CORTf of house sparrows across Mexico, and to identify factors that may be driving large-scale variation in CORT. A secondary goal was to evaluate relationships between CORTf and weather variables in this population to add to existing research exploring relationships between hormone secretion and weather conditions. One prediction was that the spatial analysis would show that CORTf levels are highest in birds living in low precipitation areas, similar to the findings of Busch *et al*. (2011). Another prediction was that CORTf would be negatively related to both elevation and latitude. High-altitude and high-latitude areas are considered to be severe or demanding environments. However based on the evolutionary history of house sparrows and their relatively recent expansion into tropical environments, tropical regions (i.e. lower latitudes and altitudes) may be more challenging for this species. Supporting this theory, house sparrows do not occur in the Yucatan peninsula, one of the more tropical areas of the country (Alemán and García 1974; Lowther and Cink 2006). That said, high altitudes or latitudes may also be challenging for sparrows, as these habitats are more demanding than average temperate areas. Final predictions were that CORTf would be negatively related to both temperatures and precipitation levels during the moult period. This relationship with temperature could reflect direct effects of temperature on energetic demands as suggested based on CORTfe data (Frigerio *et al*. 2004; Lobato *et al*. 2008). It could also be the result of indirect effects of temperature on food

availability, as suggested based on a CORTp study (Jenni-Eiermann *et al*. 2008). As has been hypothesized for CORTp-precipitation relationships (Bize *et al*. 2010; Busch *et al*. 2011), associations between rainfall and CORTf are likely indirect, mediated through effects of rainfall on food supply.

Determining how CORTf varies across house sparrows' range in Mexico and relating this variation to weather can help identify types of conditions that are energetically demanding for these birds. This important information will allow us to anticipate how populations may respond to future environmental change, and also to plan effective habitat management for conservation purposes. Furthermore, obtaining this type of baseline CORTf data is critical so that we have a frame of reference to compare house sparrow CORTf levels to in future studies. Finally, these results will add to our understanding of how temperature and precipitation affect the CORT response and determine whether previously obtained relationships between weather and CORTp or CORTfe can be reproduced using CORTf.

2.2 Materials and Methods

2.2.1 Study species and study area

House sparrows are native to Europe and Asia but were introduced to the United States in the 1850s; their North American range now extends from northern Saskatchewan and Manitoba to Panama (Lowther and Cink 2006) and they spread across the majority of Mexico between 1910 and the 1970s (Robbins 1973; Schrey *et al.* 2011). Their success as an invasive species has been partly attributed to the facts that they are generalist feeders and human commensals (Lowther and Cink 2006). The human population of Mexico has increased rapidly since the 1940s, and the percentage of the population living in urban areas increased from 35.1% in 1940 to 78% in 2010 (United Nations 2012). Although most house sparrows in Mexico likely reside

in urban areas or near rural dwellings, climate and physical geography vary considerably across the country. The coasts and the Yucatan peninsula are characterized by warmer temperatures (mean annual temperature >22 °C), while the majority of the interior is cooler (mean annual temperatures ranging from 12 – 22 °C; in some high altitude regions mean annual temperatures range from <5 – 12 °C; Alemán and García 1974; Rudolph 1985). The Sonoran and Chihuahuan deserts are located in north-central Mexico, and along with the Baja peninsula these regions are arid and subject to extreme high temperatures (mean July temperatures can range from 25-30 °C) and considerable annual temperature variability (range of 16-20 °C; Alemán and García 1974). Mean annual rainfall is highest on the Yucatan peninsula (range of 116-131 cm) and in the southern and central regions of Mexico (range of approx. 38-115 cm), while the northern plateau region and the Baja peninsula receive less annual precipitation (mean annual levels 11-27 cm and 1-10 cm, respectively; Alemán and García 1974; Rudolph 1985).

2.2.2 Field methods and weather data

Feathers were collected from December 2006 to March 2007 as part of a study that developed a feather δ^2H isoscape for Mexico (Hobson *et al*. 2009). Sampling sites (n=49) were chosen based on obtaining adequate coverage of the country and also on accessibility from roadways. Birds were captured using mist nets and individuals were sexed and assigned an age class (hatch year, HY; after hatch year, AHY; second year, SY; after second year, ASY; or unknown, U). A unique numbered aluminum band (U.S. Fish and Wildlife Service) was attached to each individual and wing length was measured (wing ruler, to the nearest mm). The number of individuals sampled per site ranged from 1 to 20, with a mean and mode of 9. Prior to CORT analysis feathers were stored in paper envelopes at the Environment Canada laboratory of Dr. Keith Hobson in Saskatoon, Canada.

At each site, latitude, longitude, elevation, and evapotranspiration were measured. Monthly precipitation, mean annual precipitation, and average monthly minimum and maximum temperatures were obtained for each site using information presented in Hijmans *et al*. (2005). Monthly deuterium excess (*d*-excess = $\delta^2H - 8\ \delta^{18}O$; Clark and Fritz 1997) was calculated for each site. Deuterium excess can be used as a proxy for evaporative conditions (Clark and Fritz 1997) and previous work showed that in Mexico, groundwater *d*-excess values are lower in semi-arid regions compared to the rest of the country (Wassenaar *et al*. 2009). This variable may supplement the analysis of CORT-precipitation relationships in terms of identifying relationships between moisture availability and hormone levels.

2.2.3 Corticosterone analysis

Most sample envelopes contained three flight feathers, P1, S1, and an outer rectrix; however, in some cases, one or more of these feathers was not collected. Due to missing feathers, secondaries were used for 438 samples and rectrices for the remaining 10 samples. CORT levels did not differ significantly based on which feather was used (Welch two-sample t-test, $p=0.22$). Prior to analysis the calamus was removed then each feather was measured to the nearest mm using a wing ruler. Feathers were measured because CORT values were reported in pg/mm, based on the hypothesis that CORT is deposited into feathers in a time-dependent rather than a mass-dependent fashion (Bortolotti *et al*. 2008; Bortolotti 2010). After measurement feathers were cut into small pieces ($<5\ mm^2$) using scissors.

Corticosterone was recovered from feathers using a methanol-based extraction technique which is fully described and validated in Bortolotti *et al*. (2008). Feather pieces were covered with 10 mL of methanol (HPLC grade, VWR International, Mississaugua, ON) and then vials were placed in a sonicating water bath at room temperature. After 30 minutes in the sonicator

samples were transferred to a 50 °C water bath and incubated for ≥12 hours. Feather pieces were then removed from the sample via vacuum filtration with a piece of synthetic polyester fibre used to plug the funnel. The original sample vial, the feather pieces, the filtration funnel, and the polyester plug were washed twice with ~5 mL of methanol, which was added to the sample. Methanol extracts were placed under a fumehood until vials were completely dry, 7-10 days, after which samples were reconstituted in 600 μL of phosphate-buffered saline (PBS; 0.05 M, pH = 7.6). To evaluate the efficiency of the recovery procedure three feather samples spiked with ~5000 CPM of ^{3}H-corticosterone (Amersham Bioscience) were included in each recovery. The 448 feather samples were recovered in 5 batches; for all batches >90% of the radioactivity was recoverable from the reconstituted samples (mean recovery efficiency 95.8%, SE ±1.28%). Final CORT values were adjusted to account for recovery efficiency. Reconstituted samples were stored in a -20°C freezer prior to radioimmunoassay.

Reconstituted samples were analyzed via standard radioimmunoassay procedures (Wayland *et al*. 2002), with each sample analyzed in duplicate. To avoid bias samples were placed in random order prior to analysis. Samples were also identified by a band number so that the investigator was blind to the site at which each sample was collected. Serial dilutions of house sparrow feather extracts were shown to be parallel to the corticosterone standard curve, indicating that there were no substances in the extracts that compromised the assay (Bortolotti *et al.* 2008; Buchanan and Goldsmith 2004). Dextran-coated charcoal was used to separate bound and unbound hormone. To evaluate assay variability, three internal standards containing known concentrations of hormone were included in each assay. Samples were processed in 10 assays, with a mean intra-assay coefficient of variation (CV) of 6.57% (range 4.50-10.6%) and an inter-assay CV of 4.63%. The mean detection limit (80% bound) was 10.0 pg CORT per 100 μL of

sample but all data values were considerably greater than this limit (mean = 43.0 pg/100 μL, range 17.2-237). All analyses were performed at the University of Saskatchewan, Canada.

2.2.4 Statistical analyses

Data exploration was conducted by following the procedure outlined by Zuur *et al*. (2010), including outlier analysis and evaluation of heteroscedasticity and collinearity of explanatory variables. The distribution of CORTf in the sample was plotted; other preliminary analyses included using t-tests and ANOVAs to explore relationships between CORTf and sex and age class, and using simple linear regressions, controlling for sex, to evaluate relationships between CORTf and wing length.

To explore spatial variation in CORTf, correlations between CORTf and latitude, longitude, and elevation were evaluated using simple linear regressions. T-tests were used to look for variation in CORTf between the Atlantic and Pacific drainage basins and between the interior and exterior regions of Mexico. ANOVA was used to test for differences in CORTf related to evapotranspiration. Simple linear regressions were used to test for one-way relationships between CORTf and deuterium excess or monthly weather variables.

Relationships between CORTf and multiple explanatory variables were explored by developing two sets of 15 candidate general linear models (GLMs). Each set included one global model that contained all explanatory variables thought to potentially explain variation in CORTf. The first set used the original weather data as explanatory variables and the second used the two NMDS axes (see below) as explanatory variables in lieu of monthly weather data. Each model contained a combination of explanatory variables chosen based on a specific prediction of which factors are important determinants of variation in CORTf. The best approximating model was chosen based on Akaike's Information Criterion (AIC), which aims to optimize the trade-off

between model fit and the number of parameters included in the model (Burnham and Anderson 1998).

To further explore the influence of weather variables on CORTf, ordination was used to reduce the dimensionality of the weather data. Weather data were non-normal and non-linear so non-metric multidimensional scaling was used as the assumptions of this method are less strict than those of more traditional ordination techniques such as principal components analysis (PCA; McCune and Grace 2002). An iterative technique, NMDS seeks to preserve the rank order of relationships among objects by finding the configuration of points in reduced space that minimizes the deviance of the rank order of points in this reduced space from the rank order of points in the original space, or to minimize "stress" (McCune and Grace 2002). The Euclidean distance metric was used as this metric produced solutions with low stress compared to other metrics considered, and a final solution with two dimensions (axes) was chosen based on the same criterion (minimizing stress). CORTf was plotted against these axes and correlations between original variables and axis scores (similar to factor loadings produced by PCA) were used to interpret the weather conditions represented by each axis.

Univariate regression trees were used to identify the most important predictors of CORTf. Regression trees recursively split a data set into mutually exclusive, binary subsets. The algorithm uses criteria defined by predictor variables to split objects based on values of the response variable, with each split based on a single predictor variable and the "best" split being the one that maximizes homogeneity of the response variable within each group (McCune and Grace 2002). Instead of setting stopping rules the tree was "pruned" by plotting tree size versus relative error then selecting the tree with the lowest error value. Each tree was run 10 times and each run included 1000 cross-validations. Cross validation error values estimate prediction error

of the model, and error averaged over all runs is reported for each tree. All statistical analyses were performed in R v. 2.14.0 and the 'mvpart' library was used for regression trees (R Development Core Team 2011).

2.3 Results

2.3.1 Distribution of CORTf and relationships with sex, age, and body condition

The majority of the individuals sampled had CORTf levels ranging from 2.5 - 10 pg/mm, but 20 individuals had higher levels. These extreme individuals did not consistently belong to one sex or age class. However, they were all sampled at one of five sites, with 11 of the extremes captured at a single site. Additionally, 19 of the 20 individuals with high CORTf values were sampled in the Atlantic drainage basin. These 19 birds had feather deuterium values in the mid-range for the sample, but the one individual with a high CORTf value (13.62 pg/mm) sampled from the Pacific drainage basin also had a high, negative feather deuterium value (-92.7 ‰; sample mean = -61.3 ‰, range = -14.1 to -94.9 ‰). CORTf levels did not differ significantly between the sexes (Males $\overline{x}$ = 5.8 pg/mm, Females $\overline{x}$= 5.6 pg/mm; p=0.42) or age classes (ANOVA, df=4, p=0.74). CORTf levels were not strongly related to wing length for either sex (Males R^2=0.007705, F=1.833, df=236, p=0.1771; Females R^2=0.0003422, F=0.06229, df=182, p=0.803).

2.3.2 One-way relationships between feather CORT and spatial or weather variables

One-way associations between CORTf and latitude, longitude, and elevation were weak (R^2 values all <0.045) although the regression with latitude was significant ($p=1.04 \times 10^{-5}$). CORTf values differed significantly between the drainage basins east versus west of the continental divide (Atlantic $\overline{x}$= 6.4 pg/mm, Pacific $\overline{x}$ = 5.1 pg/mm; $p=1.04 \times 10^{-6}$; Figure 2.1) and between the interior and exterior drainage basins, (defined as described in Wassenaar *et al*.

2009; Interior $\overline{x}$ = 6.4 pg/mm, Exterior $\overline{x}$ = 5.2 pg/mm; p=1.14 x 10^{-5}; Figure 2.1). These differences persisted after removing individuals with unusually high CORTf values (n=20) from the data set (p=0.00086 for drainage basin, p=0.023 for interior/exterior). CORTf also differed significantly based on evapotranspiration level (ANOVA, df=11, F= 4.46, p=2.19 x 10^{-6}), with higher mean CORTf values associated with low evapotranspiration values (<100 and 200-300). Regressions modelling relationships between CORTf and monthly precipitation, minimum and maximum temperature, and *d*-excess values did not reveal strong one-way associations (all R^2 values <0.08); however many models had significant p-values at the α=0.05 level.

2.3.3 Ordination and Modelling

Examining correlations between NMDS axes and weather variables showed that Axis 1 was positively associated with precipitation during the moult period (conservatively estimated to be June-November based on Mathew and Naik 1986; Lowther and Cink 2006; and Romero *et al*. 2006) which overlaps with the rainy season in Mexico (July-September). Axis 1 was also positively associated with minimum and maximum temperatures of months outside of the moult period, in the dry season. Axis 2 was negatively associated with precipitation during the moult period/rainy season and positively associated with minimum and maximum monthly temperatures during the moult period/rainy season. High CORTf values were associated with low values of Axis 1, which corresponds to dry conditions during the moult period/rainy season and cool temperatures outside of the moult period (dry season). Mid-range values of Axis 2, which correspond to intermediate temperatures and levels of precipitation during the moult period/rainy season, were also associated with high CORTf (Figure 2.2). Evidently, there is a contradiction regarding the effect of moult period precipitation levels on CORTf. Further examination shows that some high CORTf values are also associated with high values of Axis 2,

which correspond to low levels of precipitation during moult, and mid-range values of Axis 1, which correspond to intermediate levels of precipitation during the moult period.

In the first set of candidate GLMs, which used original weather data as explanatory variables, the best approximating model was the global model, with the next-best models containing only Site, all of the monthly weather variables, and weather variables from the dry season only. The difference in AIC between the top two models was >2 meaning there was no model selection uncertainty (Burnham and Anderson 1998). The second set of models, which used NMDS axes as explanatory variables, yielded similar results; the best approximating model was the global model, followed by models containing Site only, all weather variables (the 2 NMDS axes plus *d*-excess and evapotranspiration), then only *d*-excess variables.

2.3.4 Regression Tree Analysis

Regression trees were run on the data set with and without *d*-excess data. In all cases the most important variable predicting CORTf was site. To determine whether the dominance of site was driven by 6 influential sites (those from which birds with CORTf >10 pg/mm were sampled) a step-wise elimination of these sites was conducted, with regression trees run after each removal. After removing these sites, site was still the top predictor in all trees. Age, mean annual precipitation, and *d*-excess also emerged as important predictors of CORTf. For the data set excluding *d*-excess, the modal best tree size from the 10 runs considered was 3 nodes, and both splits were based on site. To explore other potentially important predictors of CORTf the variable site was removed from the analyses. Regression trees run without site identified minimum temperatures in January, June, and July, and age class as important predictors of CORTf (Figure 2.3). Re-running regression tree analyses of the data set including *d*-excess data after removing site identified *d*-excess in April and May precipitation as important predictors of

CORTf (Figure 2.4). As some of the weather variables were collinear, for the trees developed after removing site I looked at surrogate variables for each split. Generally the surrogate variables were quite similar to the variable on which the split was based. However, in some cases precipitation variables or spatial data (latitude or longitude) appeared as surrogates for temperature variables, or vice versa. Improvement values for each surrogate variable were low (all <0.35), indicating that using these variables instead would not result in a considerable increase in within-group homogeneity.

2.4 Discussion

The dominant spatial pattern in CORTf was the clustering of the high-CORT birds in the north-central region of Mexico, where average conditions are hot and dry (mean annual temperatures ranging from 12-22 °C and mean annual rainfall ranging from 11-27 cm; Alemán and García 1974). The tendency for CORTf levels to be higher in the driest parts of a species' range is similar to previous findings using CORTp (Busch *et al*. 2011). Contrary to *a priori* predictions, CORTf showed a weak, positive relationship with latitude and a complex association with elevation. Previous studies have found negative relationships between CORTp and latitude in snow buntings (*Plectrophenax nivalis*) and Lapland longspurs in Alaska (Wingfield *et al*. 1994), in willow warblers (*Phylloscopus trochilus*) and pied flycatchers (*Ficedula hypoleuca*) in Sweden (Silverin *et al*. 1997; Silverin and Wingfield 1998), and in Arctic shorebirds (Scolopacidae; O'Reilly and Wingfield 2001). These studies were conducted in temperate areas where latitude is closely linked to ambient climate conditions (From and Staver 1979; Gaskell and Morris 1979), and the same association does not necessarily exist in more tropical areas (Osborne 2012). However, northern Mexico is characterized as sub-tropical while the southern part of the country is tropical. Temperature and rainfall therefore show some association with

latitude across the country (Alemán and García 1974). Other work has shown no effect of latitude on CORTp (Lynn *et al*. 2003), CORTp-latitude relationships that varied between years (Lindström *et al*. 2005b) and positive relationships between latitude and CORTp (Wingfield *et al*. 1995; Martin *et al*. 2005). Previous studies have also found negative relationships between CORTp and elevation (Pereyra and Wingfield 2003; Bears *et al*. 2003; Li *et al*. 2008), and it is difficult to conceive of a biological explanation for why intermediate elevations would be associated with the highest CORT levels in a population.

Feather CORT levels were significantly different between the Atlantic and Pacific drainage basins, and also between the interior and coastal regions of Mexico. Interestingly, using feathers from the same birds, Hobson *et al*. (2009) found that feather δ^2H values also varied between the Atlantic and Pacific drainage basins. As precipitation appears to be a good predictor of CORTf variation, differences between the Atlantic and Pacific drainage basins may reflect differences in average rainfall between the two sides of the country. Based on this hypothesis, CORT levels should be lower in the Atlantic region, as the Gulf coast receives more precipitation than the Baja peninsula and the Pacific coast (Alemán and García 1974; Rudolph 1985). However, I found that CORTf levels were higher in birds from the Atlantic drainage basin, which suggests that the difference may reflect the fact that the majority of the high CORTf birds were sampled in the Atlantic region. Similarly, CORTf could be higher in the interior drainage basin because most of the extreme birds were sampled in the interior. However, re-running these analyses after removing individuals with unusually high CORTf values from the data set yielded significant p-values, suggesting that some unmeasured difference between these broad regions of the country is associated with CORTf variation.

As predicted, CORTf was negatively associated with temperatures and precipitation levels, but one-way associations were weak. However, the clustering of the high CORTf birds in the north-central, desert region of the country suggests that precipitation is an important ecological variable explaining variation in CORTf. Previous studies have found negative associations between CORTp and precipitation levels in white-crowned sparrows in Washington (Wingfield *et al*. 1983), in dark-eyed juncos wintering across the U. S. (Rogers *et al*. 1993), and in Alpine swifts in Switzerland (Bize *et al*. 2010). House sparrows eat grains primarily but also consume insects (Lowther and Cink 2006). As rainfall is important for plant growth and weather can influence the availability of insect prey (Murphy 1987), the CORTf-precipitation associations in this population could be driven by the effects of precipitation on food supply. Alternately sparrows could be at a risk of dehydration in extremely arid regions. In this case rainfall could be directly affecting CORTf by increasing the energetic costs of obtaining enough water to maintain homeostasis.

Low ambient temperatures have been associated with higher CORTp and CORTfe levels in multiple species and contexts, including diving petrels off the coast of South Georgia Island (Smith *et al*. 1994), Greylag geese in Austria (Frigerio *et al*. 2004), and nestling blue tits (*Cyanistes caeruleus*) and pied flycatchers in central Spain (Lobato *et al*. 2008). Except for cases in which cool temperatures occurred during storms, these associations likely reflect effects of ambient temperature on energetic requirements or food availability, rather than cool temperatures being perceived as stressful (as suggested by Jenni-Eiermann *et al*. 2008). The thermoneutral zone is the range of temperatures in which an organism does not have to expend large amounts of energy to maintain its body temperature (Hill *et al*. 2004). When temperatures fall below the lower limit of the thermoneutral zone the amount of energy required to

thermoregulate increases. For house sparrows from various locations in the U. S. the lower bound of the thermoneutral zone has been designated as 20-22 °C and the upper bound as 37-38 °C (Hudson and Kimzey 1966). During the moult period extreme minimum temperatures at the sampling sites ranged from 8.2 °C for June to 1.4 °C for November. As CORT is known to stimulate foraging behaviour (Sapolsky *et al*. 2000; Crossin *et al*. 2012) and to make energy available by promoting glucose synthesis and the breakdown of fat reserves (Toates 1995), we would expect individuals to increase CORTf levels in response to low temperatures, as seen in this population.

Site consistently emerged as the most important predictor in modelling and regression tree analyses. As weather variables were measured at the site level this could reflect the influence of weather conditions on CORTf. Alternately, CORTf may be more strongly influenced by an unmeasured variable that differs among sites, such as conspecific density or predation pressure. Other important predictors were precipitation levels early in the breeding period (April-May) and temperatures in January and during the breeding/moult period (June-July). Interestingly, CORTf was not most strongly related to weather variables during the moult period. CORTf may reflect individual energetic condition during feather growth (Fairhurst 2011). Together this suggests that conditions experienced during the wintering or breeding stages could have effects that carry-over to influence an individual's energetic stage during moult. For example, cool temperatures during the pre-breeding or breeding stages could cause birds to expend more energy to maintain their body temperature, resulting in reduced fat storage. Unless these negative effects can be countered by increased fat storage during breeding the individual would be in relatively poor condition during the feather growth period, which could result in higher CORTf. Alternately, extreme temperatures or precipitation levels earlier in the

year could determine the quantity or quality of food available during the breeding and moult periods.

The lack of considerable variation in CORTf within the sample likely occurred because individuals with extremely low or extremely high CORT values are severely compromised in their ability to cope with their environment and often do not survive (Romero *et al*. 2009). Many studies have shown that sex, age, and body condition can influence CORTp and CORTfe (ex. Bonier *et al*. 2007; Cabezas *et al*. 2007; Wilcoxon *et al*. 2011). In this sample, however, CORTf did not differ significantly between the sexes or age groups and was not significantly associated with wing length. One explanation for the uniformity of CORTf levels of birds sampled across the country could be that house sparrows are human commensals. As these birds live in close association with humans, their habitats may be fairly consistent in terms of food supply, shelter, predation pressure, and other factors affecting energetic requirements. Therefore regardless of their location in Mexico house sparrows may face similar energetic requirements, and maintain similar CORTf levels. The north-central region of the country is an exception to this hypothesis, suggesting that in this area birds are less reliant on humans, or that living with humans cannot completely buffer house sparrows from environmental conditions. Additionally, other studies have found that urban birds show CORTp differences based on sex (Bonier *et al*. 2007), age class (De Neve *et al*. 2010), and body condition (Fokidis *et al*. 2011) despite their close association with humans, so this theory does not explain the lack of these effects. It would be interesting to repeat this study using feathers collected from a species that is not human commensal. Presumably such a species would experience greater habitat variability across its range, which would likely result in stronger associations between CORTf and both spatial and weather variables.

A hypothesis that has emerged from my results and the results of Busch *et al*. (2011) is that precipitation level is one of the most important factors governing large-scale spatial variation in CORT. More studies focusing on broad patterns in CORT are needed to further validate the precipitation hypothesis, and to identify alternate variables that show strong associations with CORT. Another focus of future work could be the apparent carry-over effect, or the tendency for CORTf to be associated with weather conditions outside of the feather growth period. Nestling CORTp levels appear to reflect weather conditions experienced in the hours immediately prior to sampling and not conditions as recently as 12-24 hours prior (Bize *et al*. 2010). This finding opposes the carry-over effects hypothesis, perhaps because CORTp and CORTf reflect hormone secretion over different time periods. It would be interesting to include both blood and feather CORT measurements in future studies to expand our knowledge of how these two measurements differ and identify the specific time period during which environmental conditions influence CORT secretion.

These results show that CORTf is a valuable tool for macrophysiology, as this analysis successfully revealed broad spatial patterns in house sparrow CORT levels. Low levels of precipitation appear to require increased energetic output in this species. Based on this finding, it can be predicted that future reductions in rainfall may result in decreased individual condition, population declines, or range contractions. Additionally, populations in low rainfall regions may be more susceptible to other negative pressures such as changes in predator or prey numbers or anthropogenic disturbances. Information such as this is vital for planning effective conservation strategies, therefore future studies should explore broad CORTf variation in other species, especially those that are currently declining or threatened.

Figures

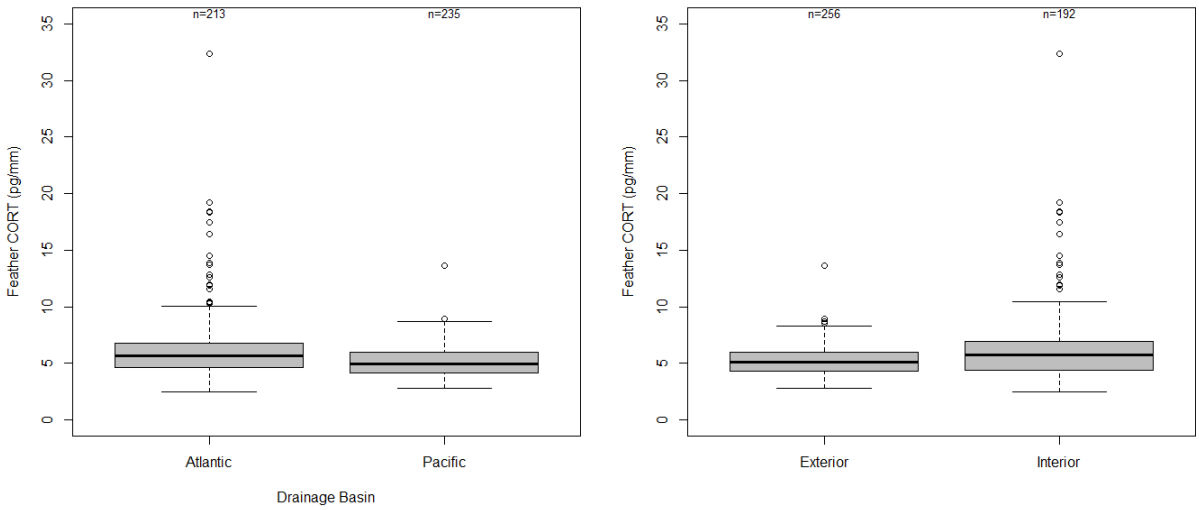

Figure 2.1: Differences in feather CORT of house sparrows sampled in different drainage basins and in the exterior versus interior regions of Mexico. The Atlantic and Pacific drainage basins are separated by the continental divide. Shown are the median (horizontal line within box), 25th and 75th percentiles (lower and upper edges of box), 10th and 90th percentiles (whiskers below and above box) and outliers (values outside the 10th and 90th percentiles; open circles). Of the 49 sampling sites, 29 were located in the Pacific drainage basin and 20 in the Atlantic drainage basin; 17 were located in the interior region and 32 in the exterior region. Welch two sample t-tests showed that feather CORT differed significantly between drainage basins ($p=1.04 \times 10^{-6}$) and also between the interior and exterior regions of the country ($p=1.14 \times 10^{-5}$).

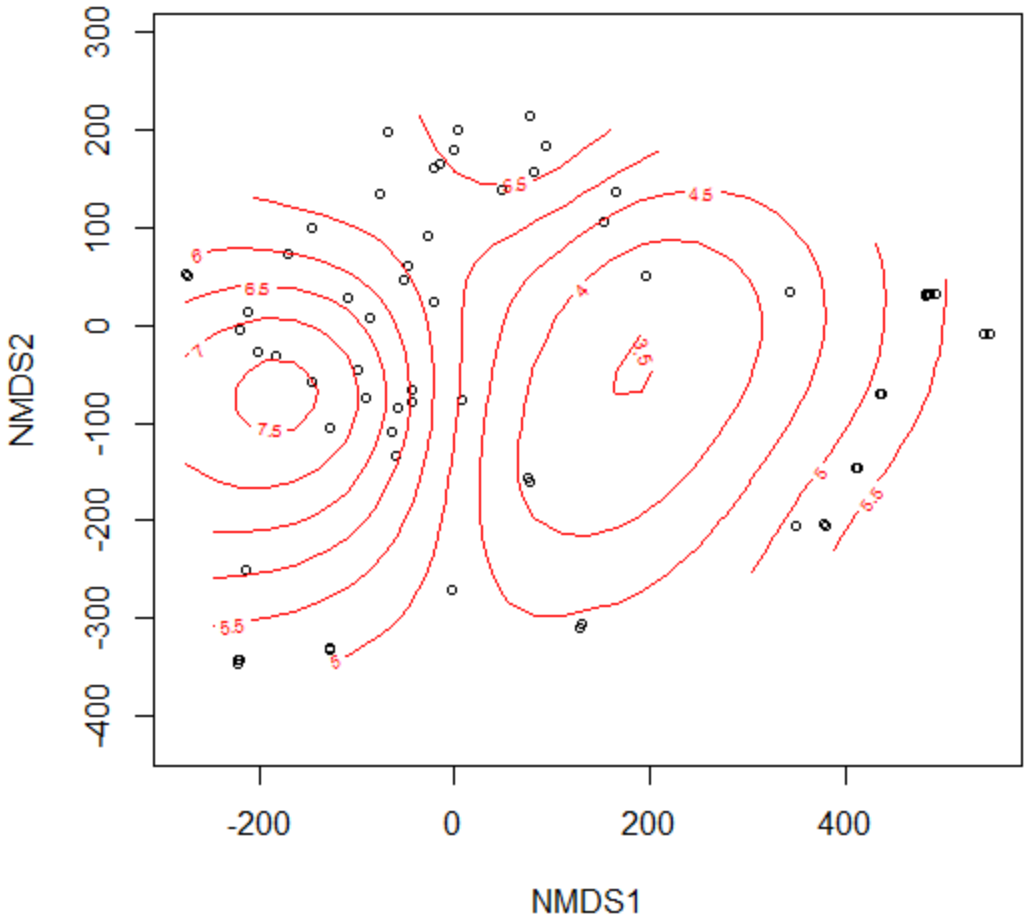

Figure 2.2: This figure shows feather CORT values of house sparrows sampled in Mexico plotted as contour lines on the reduced space created by two axes produced by ordination of 37 weather variables. Ordination was done using non-metric multidimensional scaling with a Euclidean distance metric, and the solution with the lowest stress consisted of two dimensions or axes. The first axis (NMDS1) is positively correlated with precipitation from months during the rainy season, which overlaps with the moult period of house sparrows, and positively correlated with minimum and maximum temperatures from months outside of the moult period, during the dry season. The second axis (NMDS2) is negatively correlated with precipitation during the moult period or rainy season and positively associated with minimum and maximum temperatures during the moult period or rainy season.

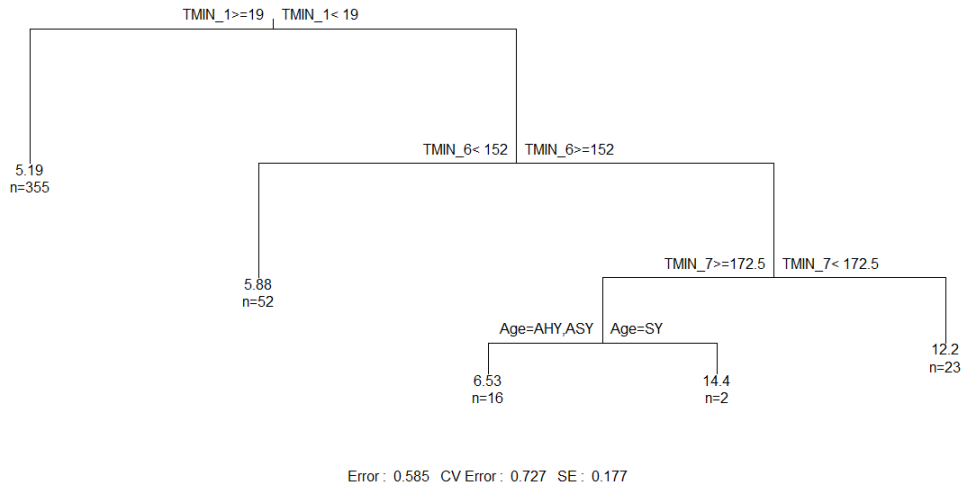

Figure 2.3: A regression tree showing the division of a sample of 448 house sparrows sampled across Mexico into groups based on feather CORT, with group divisions determined based on one of 45 predictor variables, each describing spatial location or weather conditions of sampling sites. Variables appearing near the top of the tree are more important predictors of feather CORT, meaning that based on this tree the most important variable explaining variation in feather CORT is January minimum temperature (TMIN_1) and other important variables are minimum temperatures in June (TMIN_6) and July (TMIN_7), and age class. For each terminal node the group size (n) and the mean feather CORT for that group (in pg/mm) are listed. Threshold values for each split are also listed; for temperature variables units are °C * 10. The best tree size was selected based on minimizing relative error. This model was run 10 times and each run included 1000 cross-validations. The average CV error value over all runs was 0.7709. Regression trees including the predictor variable site indicated that site is also a very important predictor of feather CORT.

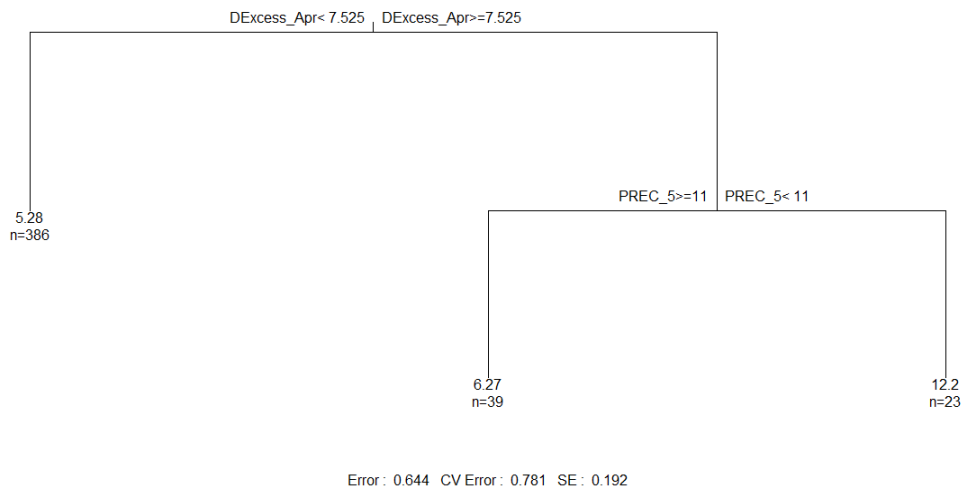

Figure 2.4: A regression tree showing the division of a sample of 448 house sparrows sampled across Mexico into groups based on feather CORT, with group divisions determined based on one of 58 predictor variables, each describing spatial location or weather conditions of sampling sites. Variables appearing near the top of the tree are more important predictors of feather CORT, meaning that based on this tree the most important variable explaining variation in feather CORT is deuterium excess in April (DExcess_Apr) and May precipitation (PREC_5; in mm) is also important. For each terminal node the group size (n) and the mean feather CORT for that group (in pg/mm) are listed. Threshold values for each split are also listed. The best tree size was selected based on minimizing relative error. This model was run 10 times and each run included 1000 cross-validations. The average CV error value over all runs was 0.6857. Regression trees including the predictor variable site indicated that site is also a very important predictor of feather CORT.

Chapter 3: Temporal variation in feather corticosterone and CORT-weather relationships in Eurasian Tree Sparrows (*Passer montanus*) in Illinois

3.1 Introduction

Long-term studies provide data that can be used to monitor population health and assess the impacts of environmental change. This type of research has long been recognized as a powerful tool in biology. Over time, human impacts on ecosystems across the globe have increased in their range and intensity. These include alteration or destruction of natural habitats for agricultural, industrial, and urban expansion and changes in global climate and biogeochemical cycles (see Vitousek *et al*. 1997). Habitat alteration and climate change have serious, negative effects on bird populations (ex. Andren 1994; Both *et al.* 2006; Lemoine *et al.* 2007), as does the human-mediated spread of invasive species (ex. Savidge 1987; Flanders *et al*. 2006; MacGregor-Fors *et al*. 2010). At any time, organisms face multiple, often inter-related challenges in their environment. It is important to understand how individuals and populations cope with these challenges so that conservation actions can be prioritized and the effects of future changes can be anticipated and potentially counteracted.

Corticosterone is the main avian glucocorticoid hormone and is involved in daily energy mobilization and in mounting a stress response when animals experience unpredictable environmental perturbations (Wingfield *et al*. 1998; Sapolsky *et al*. 2000). This measure can be considered an indicator of individual energetic state (Fairhurst 2011), and CORT levels provide insight into the well-being of organisms. More specifically, CORT can reveal whether individuals are capable of responding appropriately to environmental challenges or if they are struggling to maintain normal physiological functions. Long-term CORT sampling can help monitor the health and viability of wildlife populations and provide reference points that can be used to assess the impacts of current or future environmental changes. Despite the strong

potential of this approach, few researchers have collected CORT data over extended time periods. Diel (Rich and Romero 2001) and annual (Romero 2002) patterns of CORTp variation have been reported, and most studies measure CORT over 2-3 years, but we lack information about variation over longer time periods. One exception is a comparison of CORTf levels of great horned owls collected in 2004-05 to CORTf levels of museum specimens collected from 1931-74 which concluded that the higher CORTf levels in the museum specimens could indicate that past environmental conditions were more energetically demanding (Bortolotti *et al*. 2009a). However, the authors cautioned that these data could also reflect a bias for the individuals that were obtained as specimens to be of relatively poor quality.

The lack of long-term CORT data can be partly attributed to logistic constraints, as blood sampling and storage in the field is difficult and time-consuming. Techniques for measuring CORT metabolites from feces (Goymann *et al*. 1999; Harper and Austad 2000) and CORT from feathers (Bortolotti *et al*. 2008) mean that existing, long-term studies can now incorporate CORT sampling with relative ease. Furthermore, as CORT deposited into feathers does not degrade over time (Bortolotti *et al*. 2009a) CORTf levels of individuals preserved as museum skins can be measured.

This project explored broad-scale temporal variation in CORTf of Eurasian tree sparrows (hereafter tree sparrows) collected in Illinois from 1963-1990 that were prepared and stored as museum skins. Tree sparrows are small (~22.8 g), non-migratory human commensals native to Europe and Asia (Barlow and Leckie 2000). In 1870, about 22 individuals were introduced to St. Louis, MO; the species' current North American range is limited to west-central Illinois, extending slightly into Iowa and Missouri. Despite the lack of range expansion, the North American population of tree sparrows grew during the sampling period, from ~2,500 individuals

in 1964 to ~150,000 in 1988 (St.Louis and Barlow 1988). A more recent population estimate based on annual bird counts is 25,000 (Barlow and Leckie 2000). Current population growth and range expansion is thought to be minimal, which could be related to unsuitable habitat, competitive exclusion, or reduced reproductive success due to founder effects (Barlow and Leckie 2000). Illinois is heavily involved in agriculture and industrial activities. The climate is continental and annual weather varies considerably, with annual mean temperatures ranging from 9-14°C, annual minimum temperatures ranging from -11°C to -2°C, annual maximum temperatures ranging from 27-32°C, and mean annual precipitation levels of 810-1220 mm (Changnon *et al*. 2004). During the study period the human population of Illinois increased (U.S. Census Bureau) and habitats became more reduced and fragmented due to agricultural expansion (Iverson and Risser 1987; Iverson 1988).

The objectives of this study are to explore temporal variation in CORTf of tree sparrows in Illinois and to evaluate relationships between CORTf and weather variables. As this study is exploratory and relies on previously collected data no *a priori* hypotheses were tested, rather the goal was to explore the utility of the feather CORT technique and generate information that can inform future work. Previous work showed positive associations between conspecific density and CORTp (Nephew and Romero 2003) and CORTfe (Selva *et al*. 2011). As habitat alteration or disturbance has also been linked to CORTfe (Wasser *et al*. 1997; Thiel *et al*. 2008), one prediction was that average CORTf levels would increase over time. Another prediction was that CORTf would be negatively related to average and also extreme temperatures and precipitation levels during the moult period. Cool temperatures can increase thermoregulation costs, and increased CORT secretion helps make energy available to maintain homeostasis (Toates 1995). Accordingly, negative associations have been found between CORTp or CORTfe

and temperature in several species and systems (Romero *et al*. 2000; Frigerio *et al*. 2004; Lobato *et al*. 2008). Temperature and precipitation can also both affect CORTp indirectly through their influence on availability and quality of food resources (Jenni-Eiermann *et al*. 2008; Bize *et al*. 2010; Busch *et al*. 2011).

The results of this study will be some of the first evidence that CORTf can be used to obtain information about the physiological state of preserved birds. This ability to conduct retrospective analysis of CORT levels is groundbreaking. These data will allow us to determine how energetic challenges experienced by current populations compare to those that past populations faced, which has important implications for assessing population viability and for gauging the severity of current conditions. Identifying conditions under which past populations were not challenged is also vital, as this can help managers and conservation biologists determine what steps to take to improve existing conditions. Along with the results presented in Chapter 2, this analysis will also contribute to our understanding of how birds cope with variation in temperature and precipitation, which is important for predicting future responses.

3.2 Materials and Methods

3.2.1 Field methods, feather collection, and weather data

Field work was conducted by Dr. Jon Barlow and Dr. Vincent St. Louis from 1963-1990 (for details see St. Louis and Barlow 1988). Briefly, tree sparrows (n=433) were mist-netted in four Illinois counties (Greene, Morgan, Scott, and St. Clair). Birds were aged (adult or immature) based on skull ossification, weighed to the nearest 0.1 g, and assigned a fat class (v-light, light, light-moderate, moderate, moderate-heavy, heavy, v-heavy, or no). For 113 individuals collected from 1982-1985, 16 morphometric measurements were taken (nearest ±0.05 mm using diel calipers; St. Louis and Barlow 1987). Birds were sampled in 19 years

during the study period and sample sizes varied among years (range 1-83, mean=23 and mode=13). Sample sizes also differed between sites, however most birds (n=423) were collected from three counties within a 50 km area. Specimens were stored on dry ice prior to preparation at the Royal Ontario Museum (ROM, Toronto, Ontario, Canada). After preparation (see Section 3.2.4 for details), study skins were stored at the ROM; feathers were collected in 2009. Monthly weather data measured at the Jacksonville, IL station in Morgan County were obtained from the U. S. National Climatic Data Centre (NCDC, www.ncdc.noaa.gov). Data describing El Niño/La Niña Southern Oscillation phases and the Palmer Drought Severity Index (PDSI) were also obtained.

3.2.2 Corticosterone analysis

Corticosterone analysis was conducted as described in Chapter 2. Samples were extracted in 5 batches and for all batches >90% of the radioactivity was recoverable from the reconstituted samples (mean recovery efficiency 97.6%, SE ±1.29%); final CORT values were adjusted to account for recovery efficiency. Samples were processed in 9 assays, with a mean intra-assay CV of 5.35% (range 3.90-6.68%) and an inter-assay CV of 3.74%. Mean detection limit (80% bound) for the assays was 10.2 pg CORT/100 μL of sample but all data values exceeded this limit (mean = 66.1 pg/100 μL, range 18.0-153). All lab work was conducted at the University of Saskatchewan, Saskatoon, Canada.

3.2.3 Statistical analyses

T-tests and ANOVAs were used to explore relationships between CORTf and sex, age, site, and fat score, and relationships between CORTf and body mass were evaluated using simple linear regressions. For a subset of the data (n=113) a body size measure was developed using principal components analysis of 16 morphometric variables. Some morphometric variables

differed in scale therefore a PCA based on a correlation matrix was used because this method automatically standardizes the data. Eight individuals were missing one or more measurement, so analyses with and without missing values were compared. Removing missing values resulted in more variance being captured by the main axes and a higher R-squared value for a regression of body mass against PC1. Results obtained from this analysis are reported. Correlations with original variables showed that PC1 can be interpreted as a linear measure of body size. A linear regression of body mass on PC1 was run, and residual values were used as measures of individual condition (Gould 1975). The relationship between CORTf and body condition was explored using linear regression.

ANOVAs were used to test for effects of year and ENSO phase and simple linear regressions to explore relationships between CORTf and weather variables. Model selection and regression trees were used to identify important predictors of CORTf. The top model(s) were chosen based on AIC, which aims to optimize the trade-off between model fit and number of parameters (Burnham and Anderson 1998). Dimensionality of the weather data was reduced using ordination then a second set of candidate models were run using ordination axes as explanatory variables. Weather data were non-normal and non-linear and non-metric multidimensional scaling was used because it has relaxed assumptions compared to other ordination techniques. NMDS iteratively seeks to preserve the rank order of relationships among objects by finding the configuration of points in reduced space that minimizes the deviance of the rank order of points in reduced space from the rank order of points in the original space ("stress"; McCune and Grace 2002). Manhattan distance was used because it produced lower stress solutions compared to other distances metrics. Two axes were retained in the final solution based on comparing stress values of solutions with different dimensionality.

Correlations between original variables and axis scores were used to interpret weather conditions represented by each axis then CORTf was plotted against these axes.

Regression trees recursively split a data set into mutually exclusive, binary subsets using criteria defined by predictor variables to split objects based on values of the response variable. Each split is based on a single predictor variable and the best split maximizes homogeneity of the response variable within each group (McCune and Grace 2002). Each tree was "pruned" by plotting tree size versus relative error then selecting the tree with the lowest error value. Each tree was run 10 times with each run including 1000 cross-validations. CV error values, which estimate prediction error of the model, are reported as average values for all runs. All statistical analyses were performed in R v. 2.14.0 and the 'mvpart' library was used for regression trees (R Development Core Team 2011).

3.2.4 Feather preparation experiment

During preparation as a study skin at the ROM, each sample was treated with nothing (n=50), distilled water (n=227), water and petroleum naptha (n=3), or an aqueous solution of Palmolive green dish detergent and petroleum naptha (n=153). Treatment method was related to time, for example the water and naptha treatment was only used in 1964, detergent and naptha was the prevalent treatment from 1966-1982, and water was the only treatment used from 1983-1990 (see Table 3.1). A main goal of this study was to explore CORT differences between years therefore it is critical to ensure that changes in preparation methods are not confounding year effects. To address this issue an experiment was conducted in 2012 using feathers of 10 house sparrows (7 male, 3 female) collected in Mexico as part of a different study (for more information see Hobson *et al*. 2009 and Section 2.2.1).

Six samples per individual were used (each sample consisted of three symmetrical contour feathers; multiple feathers were used to ensure that CORTf levels would be detectable by radioimmunoassay). Each sample was randomly assigned to one of six treatment groups: no treatment, water, detergent, water and naptha, detergent and naptha, and hexane. Hexane was not one of the treatments used on the study skins but it was included in order to investigate potential effects of another organic solvent on CORTf and to corroborate previous work done by Bortolotti *et al*. (2008). The calamus was removed, feathers were measured to the nearest mm using a ruler, and feathers were cut in half along the rachis using a scalpel. Each feather half was randomly assigned to control or treatment. For each treatment there were 10 control and 10 treatment vials. Mean CORTf of treated feather halves was compared between the six groups to determine whether these treatments have the potential to extract CORT from feathers prior to analysis.

Feathers in the 'no treatment' group were not manipulated. Other treatments involved 2 minute immersions, alone or in combination, in: 10 mL distilled water, 10 mL of a mixture of 1 part green Palmolive dish detergent (Colgate-Palmolive Company, New York City, New York, U. S.) : 100 parts distilled water, 10 mL of petroleum naptha (Coleman Company, Inc., Wichita, Kansas, U. S.), or 10 mL hexane (HPLC grade, VWR International) then separating the feather pieces via vacuum filtration and leaving them to dry under a fumehood. Following immersion in the detergent solution feathers were rinsed (2 minute immersion in 10 mL distilled water followed by vacuum filtration and patting dry) twice. After treatment feather halves were extracted, reconstituted, and recovered following the procedure described in Chapter 2. All samples (n=120, 60 control and 60 treated) were extracted in one batch with a recovery efficiency of 90%. Samples were processed in 3 assays with intra-assay CV's of 9.84%, 8.45%,

and 3.37% and an inter-assay CV of 10.9%. Detection limits (80% bound) for the assays were 11.8 pg CORT per 100 μL of sample, 12.2 pg CORT/100 μL sample and 12.7 pg CORT/100 μL sample; most data values were greater than these limits (mean = 26.0 pg/100 μL, range 10.3-70.6) however 3 samples, all controls for the 'no treatment' group, did fall outside of this range (10.3, 10.4, and 11.9 pg/100 μL). ANOVA was used to compare mean CORTf values of the treated feather halves between the six treatment groups.

3.3 Results

3.3.1 Temporal patterns in feather CORT

The majority of the individuals sampled had CORTf values <13 pg/mm. Evaluation of differences in CORTf between years revealed a weak, cyclical pattern, with CORTf increasing from 1966-1970 then decreasing, increasing from 1972-1977 then decreasing, and a notable increase from 1981-84 followed by a decrease (Figure 3.1). CORTf values differed significantly between years (ANOVA, df=19, $p<2 \times 10^{-16}$), and in several analyses (described below) Year emerged as a top predictor variable.

3.3.2 Relationships between CORTf and weather variables

One-way relationships between CORTf and monthly weather or drought variables were weak ($R^2<0.1$). Variables showing stronger associations with CORTf ($R^2>0.2$) were measures of September temperatures and May drought conditions. ENSO phase during the winter prior to feather growth had no significant effect on CORTf; however there were significant effects of ENSO phase in the summer prior to feather growth ($p=2 \times 10^{-4}$) and ENSO phases of the summer ($p=0.0424$) and winter ($p<2 \times 10^{-}16$) 1 year before feather growth (Figure 3.2).

A set of 22 candidate models, including a global model, was developed with each model containing a combination of explanatory variables describing a specific prediction of which

factors are important determinants of variation in CORTf. The top model was the global model, with the two next-best models containing all of the monthly weather variables and Year only. As the difference in AIC between the top two models was >2 model selection uncertainty was not an issue (Burnham and Anderson 1998).

The ordination solution with the lowest stress consisted of two dimensions or axes. Plotting CORTf on the reduced space created by these axes showed that high CORTf values are associated with high values of Axis 1, which corresponds to high temperatures during the moult period and low levels of precipitation during the moult period and the month immediately prior to moult (Figure 3.3). High CORTf values are also associated with high values of Axis 2, which corresponds to high levels of precipitation 2 months prior to moult and high levels of precipitation near the end of the moult period. A second set of 15 candidate models using ordination axes as explanatory variables in lieu of monthly weather variables was developed. The best approximating model was the global model, with the two next-best models containing county, age, sex, and year and year only. There was no model selection uncertainty.

Regression trees were run with CORTf as the response variable and Year, Site, Age, Sex, Body mass, fat score, Feather preparation method, 112 monthly weather or drought variables, and 4 ENSO variables as predictors. Year of feather growth was identified as the most important predictor of CORTf. The modal best tree from 10 runs had 4 nodes, with two splits based on Year and one based on April precipitation. To identify other important predictors of CORTf, Year was removed from the data set and regression trees were re-run. The modal best tree over 10 runs had 13 nodes, with splits based on September temperature, May precipitation, site, August precipitation, ENSO phase of the summer 1 year prior to feather growth, ENSO phase in the winter prior to feather growth, body mass, sex, and feather preparation method (Figure 3.4).

As some weather variables are collinear surrogate variables for each split were examined. For the tree developed using the full data set measures of July or September temperatures, August precipitation levels, or drought conditions in April or May could be substituted for year. Surrogates for the split based on April precipitation were the same measure of precipitation for May, June, July, or August. For the tree developed after removing Year other potentially important predictors of CORTf are April, June, and July temperatures, precipitation levels in April, June, July, September, and October, April and May drought conditions, ENSO phase in the summer prior to feather growth and in the winter 1 year prior to feather growth, and fat score. Improvement values for most surrogate variables were low (<0.35), indicating that using these variables instead would not result in a considerable increase in within-group homogeneity. For the last split in the tree developed using the data set with Year removed improvement values were higher (0.59), indicating that similar results would have been obtained if this split had been based on precipitation in April, May, June, or August rather than feather preparation method.

3.3.3 Relationships between CORTf and other variables

Feather CORT levels did not differ significantly between sexes (Males $\overline{x}$ = 7.92 pg/mm, Females $\overline{x}$= 7.88 pg/mm; p=0.90) or age classes (Adults $\overline{x}$ = 7.87 pg/mm, Immatures $\overline{x}$= 8.02 pg/mm; p=0.62). CORTf differed significantly based on site (ANOVA, df=3, F=6.865, p=0.000159) and fat class (ANOVA, df=6, F=2.819, p=0.0106). Linear regressions of CORTf against body mass were performed for the two sexes then the two age classes separately, and although R-squared values were low (all <0.12) p-values were significant (males p=0.000725, females p=0.00622, adults p=0.011, immatures p=8.63 x 10^{-5}). In all subsets the association between CORTf and mass was positive. CORTf differed significantly based on specimen preparation method (ANOVA, df=3, F=30.23, p<2 x 10^{-16}, Figure 3.5). Due to this finding, an

experiment was performed to ensure that these treatment methods do not alter the amount of CORT recoverable from the feather (see Sections 3.2.4, 3.3.4).

The ordination of morphometric data extracted a single dominant pattern, as indicated by the high proportion of variance captured by PC1 (43.7%). Positive factor loadings for 15 of the 16 morphometric variables indicated that this axis represents overall body size, with higher PC1 values corresponding to larger birds. The variable with a negative loading on PC1, narial width, made a very minor contribution to the pattern extracted by this axis. A linear regression of body mass on PC1 showed a strong, positive association (R^2=0.5205, df=93, F=101, $p<2 \times 10^{-16}$). Residuals of this regression were used as an index of body condition, with large, negative values denoting individuals in relatively poor condition (i.e. lower than expected mass, given their body size), large, positive values representing individuals in good condition, and values near zero representing individuals in average condition. Linear regression of CORTf against this condition index revealed a weak, negative association (R^2=0.003224, df=89, F=0.2879, p=0.593; Figure 3.6). One individual had an unusually low body condition score (-4.09; median was 0.035 and maximum was 1.98); re-running the regression after removing this individual resulted in a slightly stronger, negative association (R^2=0.01524, df=88, F=1.362, p=0.246; Figure 3.6).

3.3.4 Feather preparation experiment

Two samples from the feather prep experiment were lost during the assay process, one from the 'no treatment' group and one from the 'water and naptha' group. Displacement curves produced using serial dilutions were parallel to the standard curve for each of the six treatments. For all six preparation methods the difference in mean CORTf of the control and treated feather halves was not significant. Mean CORTf values of the treated feather halves did not differ between the 6 treatment groups (ANOVA, df=5, F=2.21, p=0.067; Figure 3.7).

3.4 Discussion

The clearest temporal trend in tree sparrow CORTf was a marked increase from 1981-1984 followed by a return to relatively low levels for the remainder of the sampling period. There were also slight CORTf increases 1967 and 1977, followed by returns to lower levels in the subsequent years, which may suggest that temporal variation in CORTf is cyclical. There was no evidence of a gradual increase in CORTf over time. So while energetic challenges appear to vary among years, there is no evidence of a continual increase in energetic requirements over the sampling period.

Weather is one potential explanation for the observed pattern in CORTf. In 1981 there was a moderate drought during the moult and pre-moult periods, but conditions were normal or wetter than average from 1982-84. Temperatures were unusually high during the moult period in 1983 compared to other years during the sampling period, while spring (April-June) temperatures were unusually cool in 1982. Additionally, June-November 1982 and December 1982-May 1983 are ranked as some of the most extreme El Niño periods in the past century (National Oceanic and Atmospheric Administration Earth System Research Laboratory 2012). Therefore extreme fluctuations in weather conditions during these years could partly explain increased CORTf levels as coping with extreme temperatures is energetically demanding (Hill *et al*. 2004).

Inter-annual differences in population density, predation pressure, or food availability could also be driving variation in CORTf. However, while CORTf levels were steadily increasing (1981-84) tree sparrow population size was declining (Sauer *et al*. 2011) Tree sparrow population size increased from 1985-88, suggesting that while population density likely did not contribute to the CORTf increase, factors affecting CORTf levels may have also been limiting

population size. Interestingly, numbers of avian predators counted on BBS routes increased from 1980-81 then remained relatively steady before increasing again in 1987. Therefore increased predation pressure may have contributed to higher CORTf levels in 1981, but likely did not contribute to CORTf increases from 1982-84. However, tree sparrows also have mammalian predators such as cats and raccoons, and changes in predation pressure from these sources may have contributed to patterns in CORTf. Another potentially influential factor is food availability. In the U.S. overall, corn production was >10% lower than expected in 1970, 1974, 1983, and 1988 (Babcock 2007) and the Crop Moisture Stress Index, which measures negative consequences of moisture extremes on crop yield, was high in 1974, 1976, 1980, 1983, and 1988 (U.S. NCDC 2012). In the Illinois counties from which birds were collected the overall trend was an increase in annual corn yields over the study period but there was considerable inter-annual variability, with relatively high yields in 1979, 1981-82, 1985-87, and 1989-1990 and relatively low yields in 1964, 1966, 1970, 1980, and 1983 (U. S. Department of Agriculture National Agricultural Statistics Service 2012). Overall, the temporal pattern in tree sparrow CORTf does not line up with patterns of corn production. The low yield and the high moisture stress index in 1983 do however indicate that decreased food availability could be partially responsible for high CORTf levels in 1983-84.

As predicted, negative CORTf-weather associations emerged, the strongest being relationships with September minimum temperature and maximum precipitation in August. Previous studies have shown that CORTp elevations are associated with cool temperatures in white-crowned sparrows in Washington (Wingfield *et al*. 1983), in diving petrels off the coast of South Georgia Island (Smith *et al*. 1994), and in Greylag geese in Austria (Frigerio *et al*. 2004). High CORTp was also associated with low levels of rainfall in song wrens across the isthmus of

Panama (Busch *et al*. 2011). The relationship with temperature likely reflects increased thermoregulation costs during cooler periods, although temperature could also indirectly influence CORTp via effects on food availability (Jenni-Eiermann *et al*. 2008). The negative CORTf-precipitation relationship is likely indirectly mediated by the same pathway, especially for largely granivorous species such as tree sparrows. These CORT-weather relationships are not completely novel but this is the first time they have been shown using CORTf. Also most previous studies have focused on the breeding or wintering periods (however see Romero *et al*. 2000) and this is some of the first evidence that these relationships are found during the moult period.

Interestingly, while CORTf was negatively related to temperature in all months during the pre-moult and moult periods, CORTf was positively associated with April and May precipitation levels but negatively associated with precipitation in August. Previous studies have found CORTp elevations during cool, rainy periods and during storms involving increased precipitation in song sparrows in New York (Wingfield 1985a,b), in Lapland longspurs in Alaska (Astheimer *et al*. 1995), and in Alpine swifts in Switzerland (Bize *et al*. 2010). Similarly the positive relationship between CORTf and spring precipitation could reflect energetic challenges of inclement weather during this stage carrying over to influence individual energetic condition during feather growth. Poor weather conditions can also negatively affect foraging ability (Power 1980; Hilton *et al.* 1999) so short-term food restriction caused by high levels of precipitation may have also contributed to this effect.

CORTf tended to be higher if there was an El Niño event in the 18 months prior to feather growth, and as mentioned some of the highest CORTf values were seen in 1982-83, extreme El Niño years. While other studies have evaluated effects of extreme weather events on

CORTp (Smith *et al*. 1994; Astheimer *et al*. 1995; Raouf *et al*. 2006) this is one of the first investigations of relationships between CORT and ENSO phase. Interestingly, El Niño effects on Illinois climate are ranked as fairly mild compared to other regions of the United States, with winter conditions being slightly drier and slightly warmer than average and summers being cooler and wetter (National Oceanic and Atmospheric Administration Earth System Research Laboratory 2012). During La Niña years winters are slightly warmer and wetter, with more storms, while summers are warmer and drier. That said, the highest moult and pre-moult period temperatures seen during the sampling period occurred during the extreme El Niño event of 1982-83. This suggests that temperature extremes in either direction can be associated with increased CORT.

Relationships between CORTf and ordination axes representing weather conditions provide support for the hypothesis that the relationship between CORT and temperature is complex. CORTf was positively related to August temperature, which at first seems contradictory. However, in this case the variable is the number of days with maximum temperature ≥32.2°C, indicating that high CORTf levels are associated with both unusually cool and unusually warm periods. This is intuitive if we consider CORT to be a reflection of energetic demands, because thermoregulation costs will increase when ambient temperatures fall outside of the lower or upper bounds of the thermoneutral zone (Hill *et al*. 2004).

CORTf did not differ between the sexes or age classes, which is somewhat surprising as other studies have found sex- and age-related differences in CORTp (ex. Angelier *et al*. 2010; Heidinger *et al*. 2010; Verboven *et al*. 2010). However, many of these studies were conducted during breeding, when energetic requirements often differ considerably between the sexes. Outside of the breeding season, CORT differences based on sex or age could be related to

dominance and competition for limited resources, as social status can influence CORTp levels (reviewed in Creel 2001) and in many species adults are dominant to immatures and males are dominant to females. If this is a driver of age and sex-related CORT differences these effects would be seen primarily during times of resource limitation and high competition. Tree sparrows are human commensals (Barlow and Leckie 2000) and have access to human food sources and human-made shelter from predators and the elements. Therefore, it may be rare for this species to experience resource limitation of the severity required to necessitate strong competition. Several studies have also found no sex or age effects on CORTp (ex. Goutte *et al*. 2010; Done *et al*. 2011; Li *et al*. 2012).

CORTf differed significantly among sites, with higher CORTf levels in birds from Scott County relative to other sites. This may be an artefact of sample size differences, as considerably fewer birds were sampled from Scott (n=17) and St. Clair (n=10) counties compared to Greene (n=172) and Morgan (n=234) counties. Alternately, this effect could reflect differences in the timing of the sampling. All birds from Scott County grew their feathers in 1983, one of the years with the highest CORTf levels measured. If birds had been collected from Scott County in other years, the site effect would likely be weaker or non-existent. Scott County lies between Greene and Morgan counties. Due to this arrangement it is unlikely that some unmeasured difference in conditions between sites caused a true CORT effect; however this is a possibility.

CORTf was significantly related to fat score, but counter-intuitively birds in the 'heavy' and 'moderate' fat classes had higher CORTf levels than birds in the 'light', 'no', and 'light-moderate' classes. Previous work found no association between CORTp and fat score (Romero *et al*. 1997) but also lower CORTp levels in birds with more fat (Jenni *et al*. 2000). The lack of a

relationship in this study could simply reflect the subjectivity of assigning fat score (ex. Scott *et al.* 1995). The relationship between CORTf and body mass was positive while the relationship between body condition and CORT was negative. Other studies have found negative associations between CORTp and body condition (ex. Perfito *et al*. 2002; Long and Holberton 2004; Raja-aho *et al*. 2010). Furthermore, the body condition analysis indicates that the positive association between CORTf and mass likely reflects the fact that larger birds have higher overall energetic requirements than smaller birds and secrete more CORT to meet these demands.

The results of the feather preparation experiment indicate that variation in CORTf in the sample was likely not influenced by differences in how the feathers were treated during the preparation of the skins and the feathers used in the present study. However, it is possible that immersion or washing in certain organic solvents or other substances could strip some hormone from feathers (Bortolotti *et al*. 2008 Appendix S1), causing final CORTf values to inaccurately reflect the amount of CORT deposited into the feather during feather growth. Therefore it is critical that future studies using museum specimens account for differences in how feathers were treated, cleaned, or stored prior to CORTf analysis.

Retrospective CORT analyses using museum specimens is an exciting research avenue made possible by the feather CORT technique. Future work exploring temporal patterns in CORTf will be critical for providing data that can be used as a reference point and for exploring the normal range in CORTf variation over time, which can help determine whether recent trends in CORTf levels are cause for concern or simply represent natural fluctuations. Measuring CORTf levels in other species collected over similar time periods or in similar areas to the tree sparrow specimens could also help identify factors that may be driving the trend seen in tree sparrows. Another advantage to the feather CORT technique is the ease of collecting samples in

the field. Ongoing, long-term monitoring projects should consider collecting feathers each year so that studies similar to this one can be conducted in the future. These data could be beneficial to these projects as well, as CORTf predicted future survival probabilities of house sparrows and therefore has potential for use as a biomarker (Koren *et al*. 2012). Recent work has supported the interpretation of CORTf as a measure of overall energetic requirements (Fairhurst 2011). These data are therefore useful for determining how birds are coping with changes in their environment over time. Future work could also determine whether the CORTf-weather associations seen in this sample are repeatable in other species, particularly the finding that energetic challenges due to high levels of spring precipitation may have lasting effects on individual condition.

While it may often seem that the conditions wild birds experience have continually become more challenging over the past several decades due to increases in habitat degradation, climate change, increases in pollution and environmental contaminants, and other factors, these results indicate that this may not be the case. As long-term CORTf variation in tree sparrows was not unidirectional, it is important to identify which factors were likely responsible for lower energy requirements in years with relatively low average CORT. This project also advances our understanding of CORT-weather relationships. While weather clearly cannot be controlled, this information is important because it allows us to determine what types of weather conditions are likely to make populations more vulnerable to other negative environmental influences. For example, if extreme weather conditions such as storms or El Niño events are indeed energetically challenging, some conservation measures may be more effective if they are implemented during these periods. More work is needed to be able to inform managers, and these data show that CORTf is a highly valuable measure that will allow us to reach this level of understanding.

Tables and Figures

Table 3.1: A summary of environmental variables associated with feather CORT of Eurasian tree sparrows (*Passer montanus*) in Illinois and of feather treatment methods over the period of feather collection, 1963-1990. Year reflects the year in which the feather was grown, rather than the year of sampling. For the four variables describing El-Niño Southern Oscillation (ENSO) phase "NM" = normal, "NO" = El Niño, and "NA" = La Niña. Feather preparation methods are nothing (No), water and naptha (WN), detergent and naptha (DN), or water (W). The Palmer Drought Severity Index (PDSI) assigns a value of zero to normal conditions, a value of -2 indicates moderate drought, -3 severe drought, and positive values indicate excess rainfall (ex. +2 is moderate rainfall). Two values are reported for the May PDSI in 1964 because birds were sampled in multiple counties in that year.

Year	**1963**	**1964**	**1965**	**1966**	**1967**	**1971**	**1972**	**1973**	**1975**	**1977**	**1978**	**1979**	**1980**	**1981**	**1982**	**1983**	**1984**	**1985**	**1986**	**1989**
ENSO summer	NM	NA	NO	NM	NM	NA	NO	NM	NM	NM	NM	NM	NM	NM	NO	NM	NM	NM	NM	NM
ENSO winter	NM	NM	NA	NO	NM	NA	NM	NO	NM	NM	NM	NM	NM	NM	NM	NO	NM	NM	NM	NA
ENSO previous summer	NM	NM	NA	NO	NM	NM	NA	NO	NA	NM	NM	NM	NM	NM	NM	NO	NM	NM	NM	NA
ENSO previous winter	NM	NM	NM	NA	NO	NM	NA	NM	NA	NA	NM	NM	NM	NM	NM	NM	NO	NM	NM	NM
Feather Prep method	No	No, WN	No	No, DN	No	No	DN	DN	DN	DN	DN	No, DN	DN, W	DN	W	W	W	W	W	W
Sept. min. temp. (°F)	41	36	39	39	38	37	38	41	37	44	39	37	39	41	39	33	31	37	35	29
Days in May with ≥0.1 in precip.	6	8	6	8	11	9	8	8	11	5	9	3	5	7	8	9	7	6	9	8
August extreme max. daily precip. (inches * 100)	265	216	187	167	117	152	187	82	160	117	68	43	134	173	82	33	68	149	78	66
Days in April with ≥0.1 in precip.	5	8	8	11	12	2	7	7	5	6	12	6	7	10	7	11	10	4	7	8
May PDSI	-0.34	-1.55 -3.03	-3.26	1.27	1.02	-1.06	-0.75	2.53	3.18	-0.81	2.07	-0.69	-2.84	0.92	2.21	4.39	3.03	1.26	-2.27	-1.32

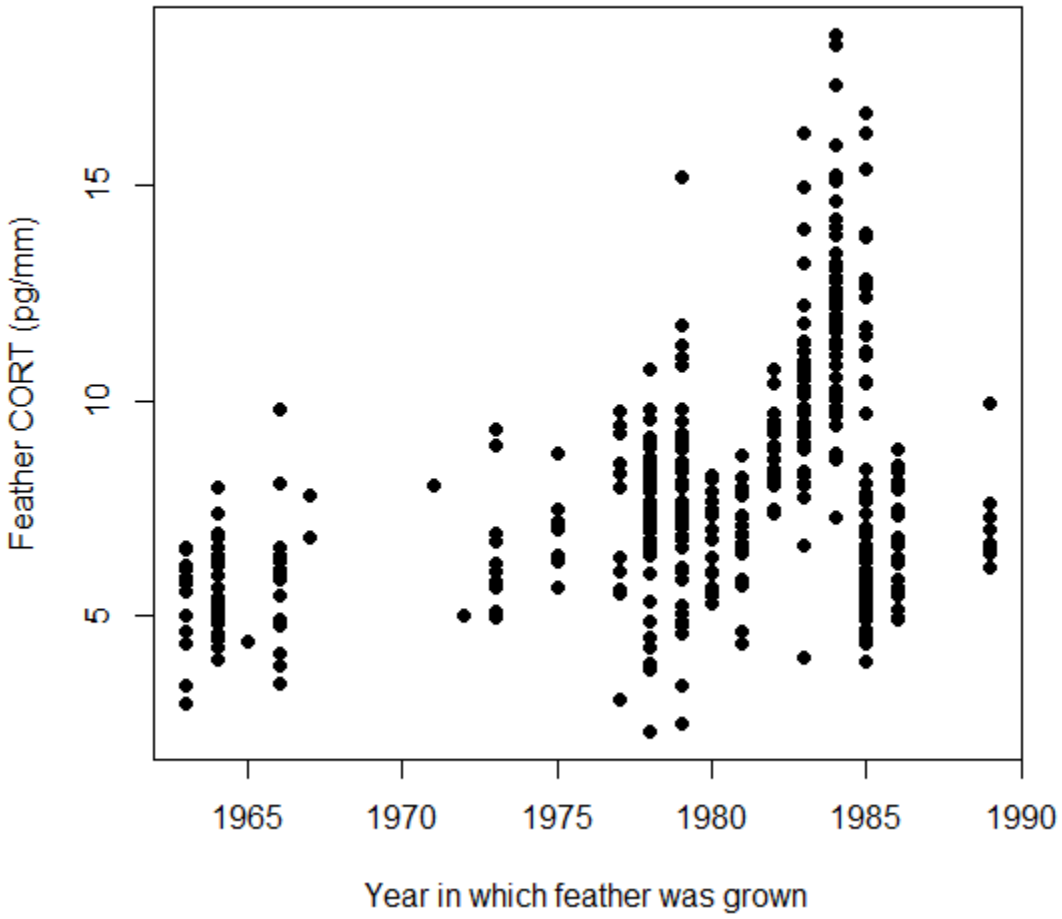

Figure 3.1: This plot shows the relationship between feather CORT and the year of feather growth in a sample of 433 Eurasian tree sparrows collected in 19 different years from four counties in Illinois. Feather CORT values differed significantly between years (ANOVA; $p<2 \times 10^{-16}$).

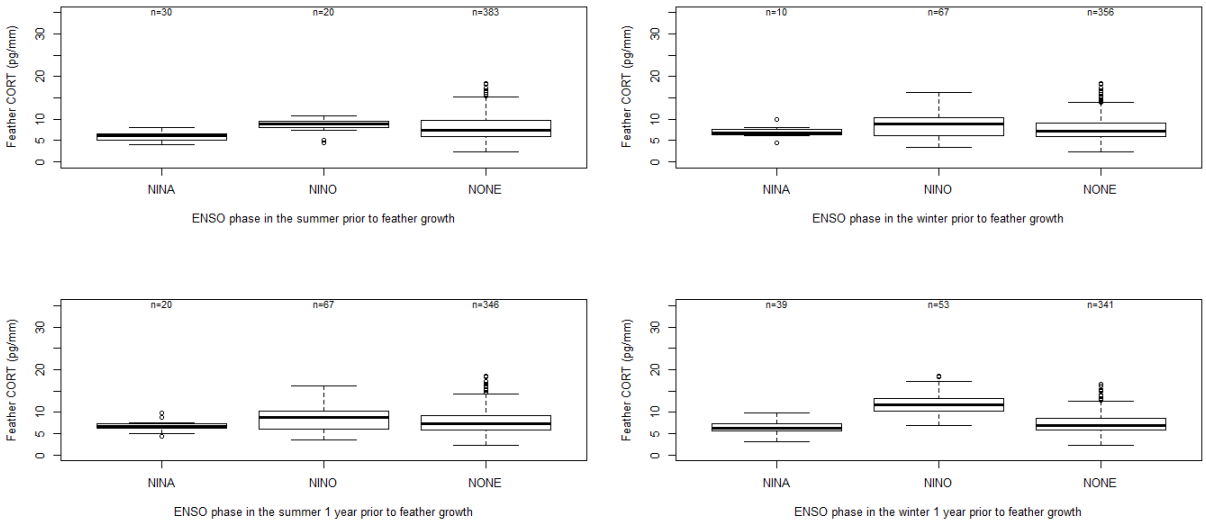

Figure 3.2: Differences in feather CORT of Eurasian tree sparrows sampled four counties in Illinois from 1963-1990, based on the phase of the El Niño/La Niña Southern Oscillation (ENSO) during summer and winter seasons preceding feather growth. Shown are the median (horizontal line within box), 25th and 75th percentiles (lower and upper edges of box), 10th and 90th percentiles (whiskers below and above box) and outliers (values outside the 10th and 90th percentiles; open circles). In Illinois El Niño summers are cooler and wetter while winters are warmer and drier; La Niña years are characterized by warmer, drier summers and warmer, wetter winters. ANOVAs showed that ENSO phase in the winter prior to feather growth was not significant; however feather CORT differed significantly based on ENSO phase in the summer prior to feather growth ($p=2 \times 10^{-4}$), ENSO phase of the summer 1 year prior to feather growth ($p=0.0424$), and ENSO phase of the winter 1 year prior to feather growth ($p<2 \times 10^{-16}$).

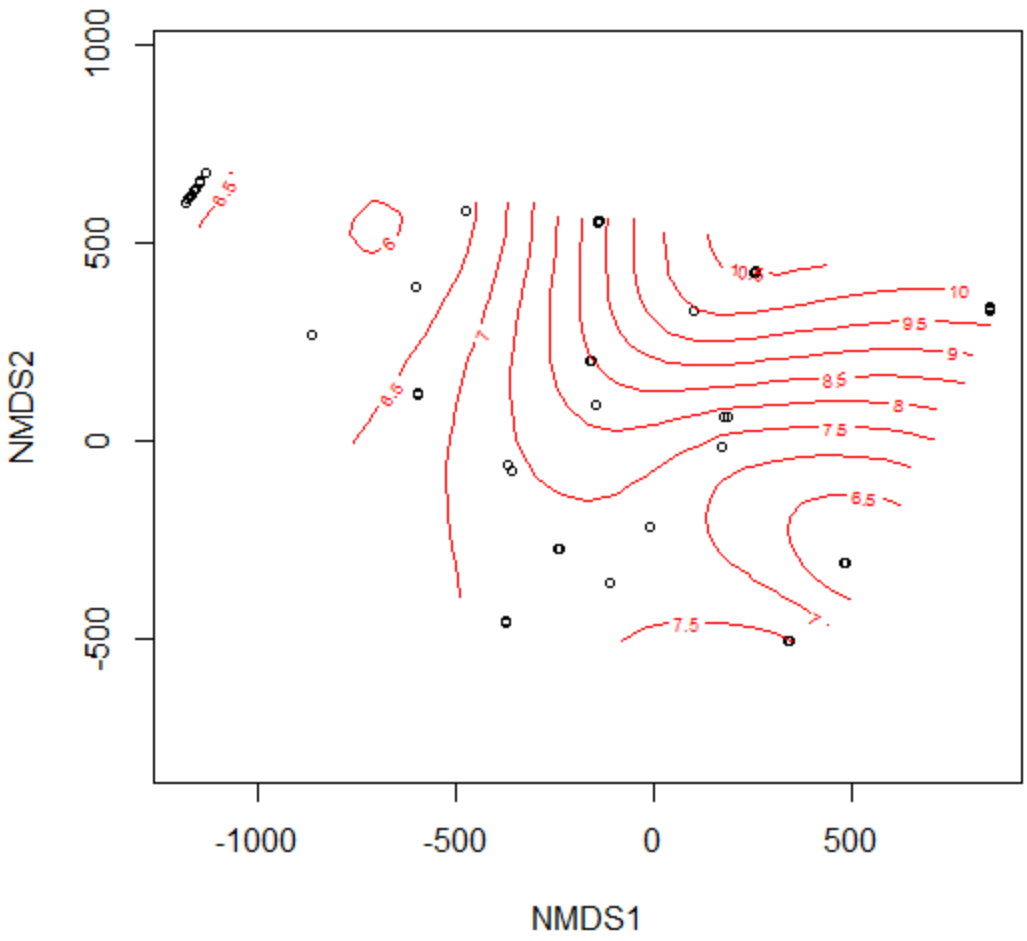

Figure 3.3: Feather CORT values of Eurasian tree sparrows sampled in Illinois from 1963-1990 plotted as contour lines on the reduced space created by two axes produced by ordination of 105 weather variables. Ordination was done using non-metric multidimensional scaling with a Manhattan distance metric, and the solution with the lowest stress consisted of two dimensions or axes. The first axis (NMDS1) is positively correlated with August temperature and negatively correlated with precipitation levels in June and July. The second axis (NMDS2) is positively correlated with precipitation levels in May and September. Contours indicate that high feather CORT levels are associated with high August temperatures and high levels of precipitation in May and September.

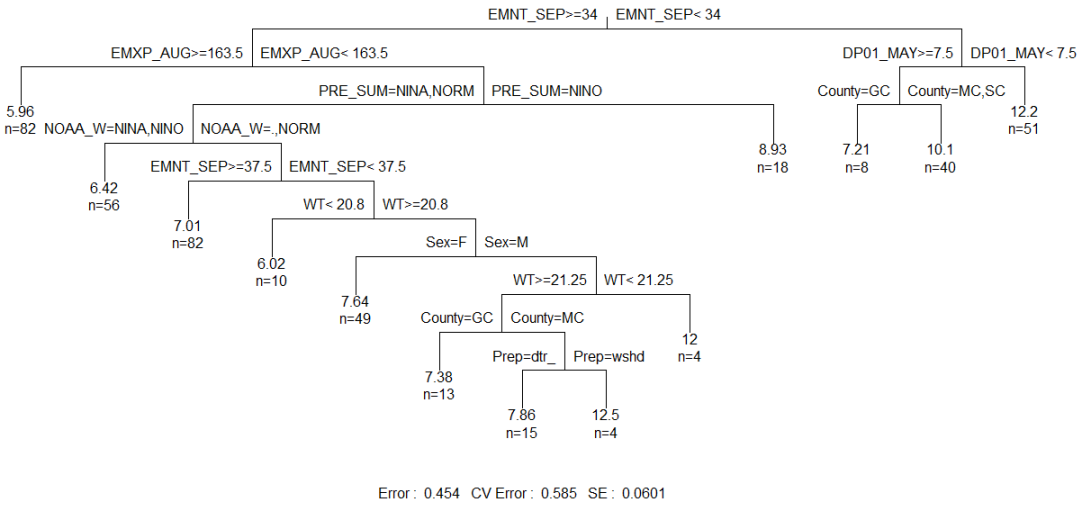

Figure 3.4: A regression tree showing the division of a sample of 433 Eurasian tree sparrows sampled in Illinois from 1963-1990 into groups based on feather CORT, with group divisions determined based on one of 123 predictor variables, including monthly weather conditions and variables such as age, sex, and body mass. Variables appearing near the top of the tree are more important predictors of feather CORT, meaning that based on this tree the most important variable explaining variation in feather CORT is extreme minimum temperature in September (EMNT_SEP; in °F) and other important variables are the number of days in May with ≥0.1 inch precipitation (DP01_MAY), County (site), extreme daily maximum precipitation in August (EMXP_AUG; in inches * 100), ENSO phase in the summer 1 year prior to feather growth (PRE_SUM), ENSO phase in the winter prior to feather growth (NOAA_W), Body mass (WT; in g), Sex, and feather preparation method (Prep). For each terminal node the group size (n) and the mean feather CORT for that group (in pg/mm) are listed. Threshold values for each split are also listed. Branch lengths were set at a uniform size so that all labels could be displayed clearly, therefore the length of each branch does not represent the proportion of variance explained by a given predictor variable. The best tree size was selected based on minimizing relative error. This model was run 10 times and each run included 1000 cross-validations. The average CV error value over all runs was 0.5719. Regression trees including the predictor variable year indicated that year of feather growth is also a very important predictor of feather CORT.

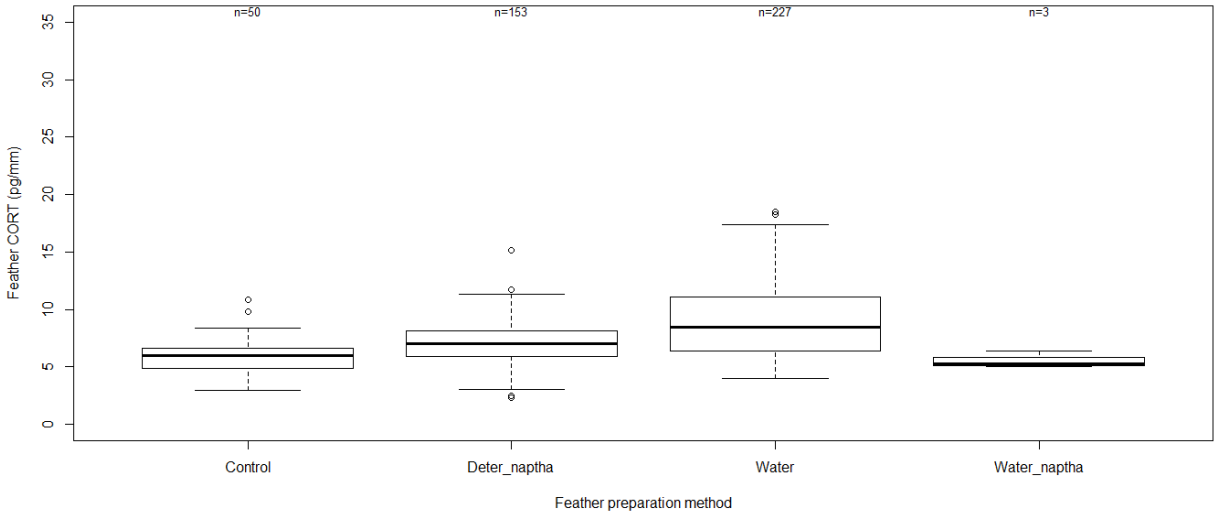

Figure 3.5: Differences in feather CORT of Eurasian tree sparrows based on feather preparation method. The codes correspond to four different methods for preparing specimens as study skins ('Deter_naptha'= washing with dish detergent, then with petroleum naptha, 'Control'= no treatment, 'Water'= washing with distilled water, 'Water_naptha'= washing with distilled water, then with petroleum naptha). Shown are the median (horizontal line within box), 25th and 75th percentiles (lower and upper edges of box), 10th and 90th percentiles (whiskers below and above box) and outliers (values outside the 10th and 90th percentiles; open circles). An ANOVA showed that feather CORT differs significantly between treatments ($p<2 \times 10^{-16}$).

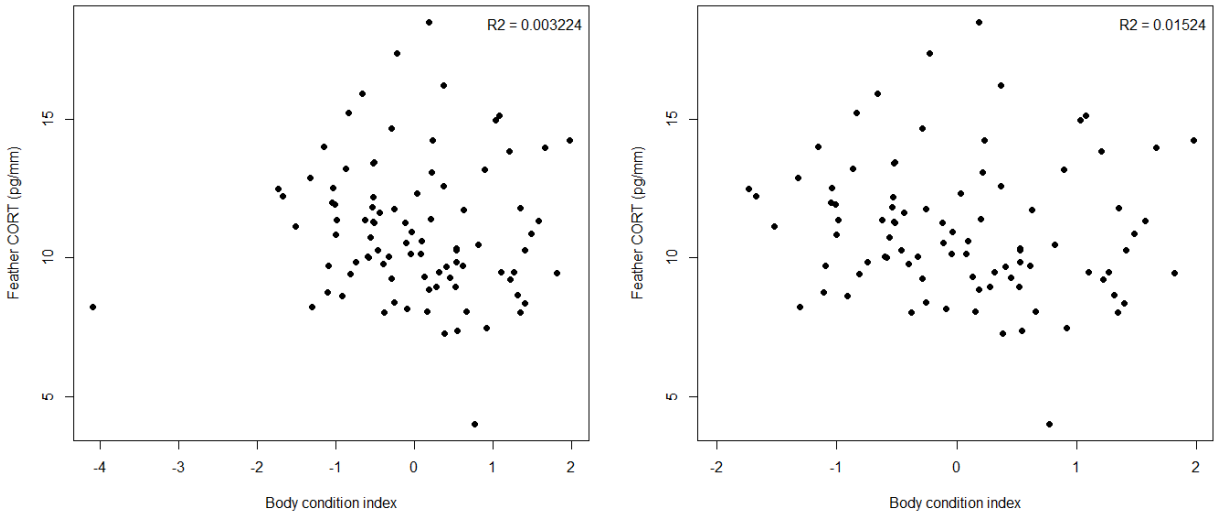

Figure 3.6: The relationship between feather CORT and body condition of Eurasian tree sparrows collected in Illinois from 1982-1985. The body condition index is the residuals of a linear regression of body mass against PC1, a measure of overall body size developed by performing a PCA of 16 morphometric variables. The figure on the left (n=95) includes one individual with an unusually low body condition value (-4.09) and the figure on the right (n=94) shows the same relationship with this extreme bird removed. Neither regression was significant (with extreme p=0.593, without extreme p=0.246).

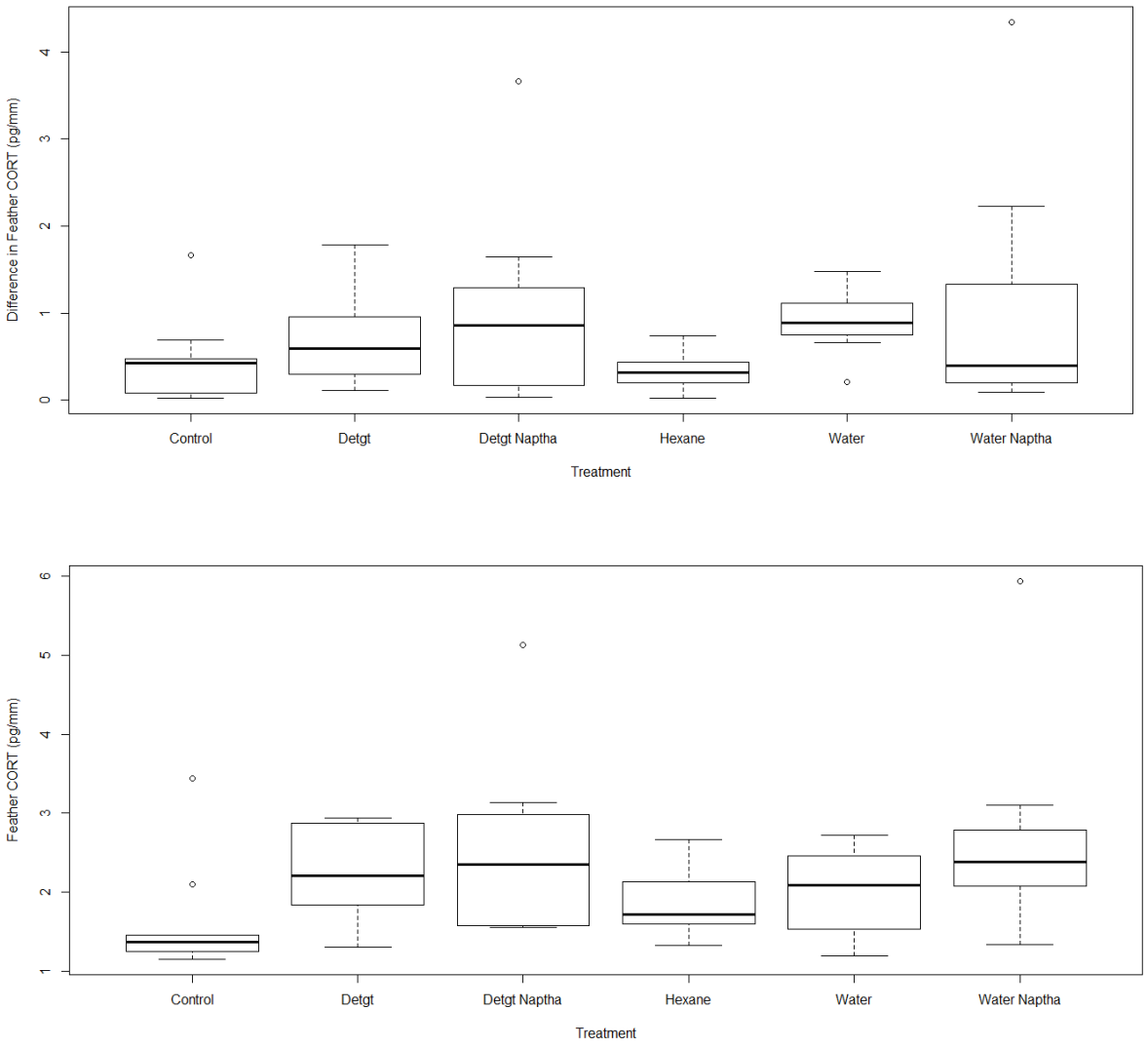

Figure 3.7: Results of an experiment designed to test the effects of 6 feather preparation methods on feather CORT values. For each treatment feathers from 10 individuals were cut in half along the rachis and one half of each feather was treated while the other half acted as a control. The treatments involved soaking feather halves for 2 minutes in: dish detergent diluted in water (Detgt), dish detergent diluted in water followed by petroleum naptha (Detgt Naptha), hexane (Hexane), nothing (Control), distilled water (Water), and distilled water followed by petroleum naptha (Water Naptha). Shown are the median (horizontal line within box), 25th and 75th percentiles (lower and upper edges of box), 10th and 90th percentiles (whiskers below and above box) and outliers (values outside the 10th and 90th percentiles; open circles). The top panel shows the difference in feather CORT between the control and treated feather halves for each sample (absolute values); the bottom panel shows feather CORT values of the treated feather halves only. Mean feather CORT of the treated feather halves did not differ significantly between the 6 treatment groups (ANOVA, df=5, F=2.21, p=0.067).

Chapter 4: Discussion

4.1 Conclusions regarding broad-scale variation in feather CORT and relationships with weather

The dominant pattern that emerged from the analysis of spatial variation in CORTf of house sparrows across their range in Mexico was that the individuals with the highest CORTf levels occurred in the desert-like, north-central region of the country. This corroborates past evidence that individual CORTf levels are a reflection of the overall energetic demands an individual faces in their environment (Bortolotti *et al*. 2008; Fairhurst 2011). Contrary to *a priori* predictions there were no strong associations between CORTf and either latitude or elevation. While previous studies found that CORTp levels often show negative relationships with latitude and elevation (Silverin *et al*. 1997; Silverin and Wingfield 1998; Pereyra and Wingfield 2003; Li *et al*. 2008), this work was conducted in temperate areas where latitude and climate are strongly linked (From and Staver 1979; Gaskell and Morris 1979). In tropical regions climatic conditions tend to be less variable overall and show weaker associations with latitude (Osborne 2012) so it is not altogether surprising that latitude did not strongly affect CORTf in this population. However, the link between climate and elevation exists in both temperate and tropical areas (From and Staver 1979; Osborne 2012). Therefore, it was surprising that CORTf was only weakly related to elevation. One possible explanation for this result is that house sparrows may be buffered from extremes in ambient conditions because they exploit human sources of food and shelter.

Other spatial patterns that emerged were that CORTf levels were significantly higher in birds sampled from the Atlantic drainage basin versus the Pacific, and also significantly higher in birds sampled from the Interior versus the Exterior region of Mexico. These differences persisted after removing 20 individuals with unusually high CORTf values from the data set,

suggesting that energetic requirements for house sparrows differ between these broad regions of the country. These regional differences could be related to weather conditions, as some weather variables considered in this study were important predictors of CORTf. Differences between drainage basins and the Interior versus Exterior could also be related to an unmeasured variable such as conspecific density, predation pressure, food availability, or environmental contaminants.

The strongest temporal trend in CORTf of Eurasian tree sparrows in Illinois was a considerable increase in average CORTf levels from 1981-84 followed by a decrease to lower levels for the remainder of the sampling period (1985-1990). Compared to this trend there was little variation in CORTf levels during the 1960s and 1970s, although it could be argued that there was a weak cyclical pattern in the data with CORTf levels increasing over 4-5 years then decreasing for 2-4 years before showing another increase. Several weather variables emerged as strong predictors of CORTf and in 1982-1983 there was a severe El Niño event and extreme cool and warm temperatures were recorded relative to the other study years. Thus, temporal trends in CORTf could be driven by inter-annual variation in weather conditions. The period of CORTf increase coincided with a decline in the Illinois tree sparrow population (Sauer *et al*. 2011) so conspecific density was likely not a contributing factor. However, this information supports the conclusion that tree sparrows faced considerable energetic challenges during this period. An increase in avian predator numbers in the early 1980s may have contributed to the CORTf increase in the first 1-2 years, and though the continued increase was likely not attributable to changes in avian predation pressure numbers of mammalian predators may have increased during this period. Finally, in 1983 moisture conditions had a strong, negative effect on annual corn yields in the study area. Decreased food availability may have also contributed to increased energetic challenges and thus increased CORTf. CORTf reflects the total energetic requirements

an individual faces during feather growth (Bortolotti *et al*. 2008). Therefore, several of these factors, in addition to other, unmeasured variables, may have acted synergistically to cause the increase in average CORTf during the early 1980s.

In both data sets CORTf was negatively associated with monthly measures of both temperature and precipitation. In tree sparrows, ENSO conditions over the two years prior to feather growth also emerged as important predictors of CORTf. Interestingly, in both data sets CORTf was associated with weather variables measured both during and prior to the period of feather growth, and conditions during feather growth were not always stronger predictors of CORTf levels. This was unexpected based on the interpretation of CORTf as a reflection of individual energetic state during the feather growth period. However, these findings indicate that individual energetic condition at any one point in time is influenced not only by current demands and conditions but also by conditions or challenges experienced in the past. This idea is similar to the concepts of seasonal interactions and carry-over effects often studied in migratory species (ex. Marra *et al*. 1998; Webster and Marra 2005; Norris 2005). The results of this study suggest that in non-migratory species challenges experienced during one stage of the annual cycle can influence physiological responses in subsequent stages.

Previous work focusing on spatial CORT variation has largely been driven by the 'short season hypothesis', which states that because birds breeding at high-latitude or high-altitude locations are exposed to frequent perturbations, have a short period of time in which to breed, and have limited re-nesting oppourtunities, they will down-regulate the HPA axis so that CORT elevations do not interfere with reproduction (Wingfield 1994). In keeping with this prediction, negative relationships between latitude and CORTp have been found several species and contexts, including in breeding pied flycatchers and willow warblers in Sweden (Silverin *et al*.

1997; Silverin and Wingfield 1998) and in breeding redpolls (*Acanthis flammea*) in Alaska (Wingfield *et al*. 1994). However, there was no difference in CORTp levels of mid-latitude versus high-latitude breeding birds in the *Calcarius*/*Plectrophenax* clade (Lynn *et al*. 2003), in house finches (*Carpodacus mexicanus*) in the eastern U. S. CORTp-latitude relationships varied between years (Lindström *et al*. 2005b), and positive CORTp-latitude relationships were found in male bush warblers (*Cettia diphone*) in Japan (Wingfield *et al*. 1995) and in house sparrows breeding in New York versus Panama (Martin *et al*. 2005). Martin *et al*. (2005) suggested that in some cases latitudinal CORTp variation may be driven by differences in parasite or disease pressure between locations rather than by variation in the length of the breeding season, explaining why their results contradicted the short season hypothesis. Similarly, in house sparrows in Mexico the weak, positive relationship between CORTf and latitude could indicate that birds living at lower latitudes experience greater parasite and disease challenges and are down-regulating CORT secretion to maintain appropriate immune system responsiveness.

A negative relationship between acute CORTp levels and altitude was found in Dark-eyed juncos (*Junco hyemalis oregonus*) in Alberta (Bears *et al*. 2003) and house sparrows from a high-altitude site in China had lower baseline CORTp then low-altitude conspecifics in Arizona, though acute CORTp did not differ between sites (Li *et al*. 2008). While these results suggest that demanding, high-altitude environments can be associated with down-regulation of the CORT response, the positive relationship between CORTf and elevation in house sparrows in Mexico indicates that these birds respond to the increased energetic challenge by increasing CORT secretion. This effect was weak, which could indicate that, as suggested by Li *et al*. (2008), birds that exploit human sources of food and shelter do not down-regulate the CORT

response based on latitude or altitude because their close association with humans ameliorates the severity and unpredictability of their environment.

This study is novel in that it was one of the first high-resolution analyses of spatial variation in CORT levels, the first examination of temporal variability in CORT over a period of >5 years, and it used an innovative measure of CORT from feathers. The only other high-resolution study of spatial variation in CORT to date showed that song wrens on the isthmus of Panama have higher baseline CORTp levels and lower body condition in low rainfall areas near their range limit (Busch *et al*. 2011). Similarly, house sparrows had higher CORTf levels in a low rainfall area in north-central Mexico, adding support to the prediction that precipitation is an important predictor of broad spatial differences in baseline CORT levels. A long-term comparison of CORTf levels of great horned owls collected in 2004-05 to CORTf levels of museum specimens collected from 1931-74 found that CORTf levels were higher in the museum specimens, suggesting that past environmental conditions may have been more energetically demanding (Bortolotti *et al*. 2009a). Similarly, in tree sparrows in Illinois the temporal pattern in CORTf over 27 years was not unidirectional, suggesting that in some cases environmental conditions fluctuate temporally rather than becoming steadily more challenging.

Previous studies have found negative relationships between CORTp and both temperature and precipitation in multiple contexts, including in song sparrows in New York (Wingfield 1985a,b), in barn swallows (*Hirundo rustica*) in Finland (Raja-aho *et al*. 2010), and in captive starlings (de Bruijn *et al.* 2011). These studies have largely been conducted in temperate areas and have used blood or fecal hormone measures. This study showed that the same CORT-weather associations are seen in house sparrows in a more tropical region, and also that these relationships can be detected in both temperate and tropical species using the feather CORT

measure. Bize *et al*. (2010) found that in nestling alpine swifts in Switzerland baseline CORTp was significantly related to conditions in the morning (5 hours) prior to sampling but not to conditions during the night (6-17 hours) or the day (18-41 hours) prior to sampling, suggesting that CORT responses to weather occur over very short time-scales. In contrast, the results of this study suggest that CORTf reflects physiological responses to weather conditions experienced both during feather growth and also in previous months. This discrepancy could reflect differences between the two measures, and it is intuitive that the instantaneous, blood measurement would reflect immediate conditions while the integrated measure of CORT from feathers provides a more holistic perspective on the many factors influencing energetic condition during the weeks in which feathers are grown. Alternately, the contradictory findings could simply indicate that the factors governing CORT secretion vary between nestlings and adults (Sapolsky and Meaney 1986; Wada *et al.* 2009).

4.2 Comparison of the two data sets

In both data sets the majority of the individuals had CORTf values in the 2.5 – 10 pg/mm range. However, only 20 house sparrows had values >10 pg/mm while 88 tree sparrows had CORTf values in this higher range. In both data sets individuals were sampled over a wide range of conditions, but the lack of considerable variation in CORTf may merely indicate that there is a narrow range of CORTf levels in which birds are healthy enough to survive to be sampled (i.e. these values likely all fall within the 'normal reactive scope' for these species, Romero *et al*. 2009). The two species examined have many similarities: they are congeners, they are similar in size and appearance, they have similar food sources and predators, they are both non-migratory human commensals, and they were both introduced to the areas in which they were sampled. However, these species showed very different post-introduction population trajectories. House

sparrows have expanded their range to cover most of North America but this population has been declining since the 1960s (Lowther and Cink 2006). In contrast, tree sparrows have maintained a restricted distribution but have seen population growth over the past 50 years (Barlow and Leckie 2000).

As conditions in Illinois are more similar to the temperate areas in which house sparrows and tree sparrows evolved compared to Mexico, it could be predicted that tree sparrows would tend to have lower CORTf levels than house sparrows. Additionally, as inter-annual variability in conditions in Illinois is likely less severe than variability in conditions across the country of Mexico, it would be predicted that tree sparrows would show less variable CORTf levels. In fact, tree sparrows tended to have higher (tree sparrows $\overline{x}$ = 7.90 pg/mm, house sparrows $\overline{x}$ = 5.72 pg/mm) and more variable CORTf levels than house sparrows. One explanation for the low variability in house sparrow CORTf is that as human commensals they are buffered from ambient conditions to an extent, and therefore the variability in conditions that the birds actually experience is less severe than the variability in ambient conditions across Mexico. However, tree sparrows are also human commensals; therefore this explanation fails to explain the discrepancy between the two species. In light of the different invasion histories of the two species, house sparrows could be better able to cope with a wide variety of environmental challenges. For example, if house sparrows show more flexibility than tree sparrows in their behaviour or in the food sources or shelters they use, they could be able to thrive in a wider variety of conditions or circumstances while avoiding an increase in energetic requirements, and a corresponding increase in CORTf levels.

For both data sets it was predicted that CORTf levels would be negatively related to measures of temperature and precipitation. These associations were seen for both species though

all one-way associations between CORTf and monthly weather variables were weak (low R^2 values). Interestingly, the strongest regression results for tree sparrows were with September temperature and May precipitation, while for house sparrows CORTf was most strongly associated with temperatures in December-March. Plotting CORTf against ordination axes describing weather conditions indicated that in house sparrows CORTf was associated with precipitation during moult and temperatures outside of the moult period, while in tree sparrows CORTf was associated with temperature during moult and precipitation during and prior to moult. In both analyses, there was a pattern for precipitation variables to be important predictors of CORTf for tree sparrows and for temperatures outside of the feather growth period to be most important for house sparrows.

For both data sets a model-selection approach to determining which weather variables were most influential was not very useful, as in both cases the top model was the global model, followed by models with all of the weather variables and models containing only one dominant predictor (year for tree sparrows, site for house sparrows). Initial regression tree analyses also indicated that year and site appeared to be 'swamping out' other predictor variables by capturing the majority of the variation in the data sets. However, in the initial regression tree with the tree sparrow data April precipitation was also a top predictor. After removing the dominating predictor variable from each data set, second sets of regression trees identified weather variables that were important predictors of CORTf: January, June, and July temperatures and April and May precipitation for house sparrows, and September temperature, May and August precipitation, and ENSO conditions for tree sparrows.

Overall, temperature and precipitation during feather growth were associated with CORTf in both data sets. There appeared to be a stronger 'carry-over effect' in house sparrows,

with temperatures several months prior to moult also showing relatively strong associations with CORTf. Although a similar effect was seen in tree sparrows with precipitation in the 1-2 months prior to moult, this relationship only emerged in one method of analysis. Relationships between CORTf and temperature were negative for both species, although tree sparrows did also show a positive association between CORTf and extreme, high temperatures in August; but while house sparrows showed negative relationships between CORTf and precipitation in all months, tree sparrow CORTf levels were positively associated with April and May precipitation levels and negatively associated with August precipitation levels. As discussed in Chapter 3 the change in direction of this relationship in tree sparrows could be a result of inclement weather in spring increasing energetic requirements. Heavy rainfall can make foraging difficult, and if birds get wet they may suffer increased flight and maintenance costs (Kelly *et al.* 2002; Ortega-Jimenez and Dudley 2012). These increased costs during spring could have persisted to affect energetic condition during feather growth. Heavy precipitation during the rainy seasons in Mexico likely represents more challenging conditions than spring precipitation in Illinois, so it is surprising that a positive CORTf-precipitation association was seen in tree sparrows but not in house sparrows. These results could be related to temperature-precipitation interactions, as cool temperatures and rain likely occur concurrently in Illinois more often than in Mexico.

The two species were sampled in vastly different areas and experience different average conditions. While in more tropical regions of Mexico climate variability is less severe than it is in Illinois (Alemán and García 1974; Changnon *et al.* 2004), compared to Illinois conditions in Mexico are far more different from the conditions where these species evolved (temperate regions of Europe and Asia). Also, the range of conditions experienced across the entire country of Mexico will be more variable than the range of conditions experienced between years in

Illinois. House sparrows also established in Mexico 40-100 years later than tree sparrows established in Illinois. As house sparrows have had less time to adjust to conditions in Mexico they may be more likely to encounter challenging weather conditions compared to tree sparrows in Illinois. This difference should be especially pronounced during the feather growth period, as in Mexico this overlaps with both the rainy season and the warm, summer months and in Illinois weather during the moult period is relatively mild compared to other times of the year (Alemán and García 1974; Changnon *et al.* 2004).

Based on this assumption, it would be predicted that CORTf-weather associations would be stronger in house sparrows versus tree sparrows. However, one-way associations between CORTf and monthly weather variables were actually weaker (lower R^2 values) in house sparrows compared to tree sparrows. As mentioned, this could help explain why house sparrows have been a more successful invasive species. House sparrows may be highly flexible in terms of physiological and behavioural responses to environmental challenges and therefore show less pronounced CORT elevations than tree sparrows under identical conditions. In this case, part of the reason why tree sparrows have not expanded their range to the same extent as house sparrows could be that they lack the level of physiological and behavioural plasticity necessary to cope with a wide range of environmental challenges.

4.3 Future research directions

The main hypothesis emerging from the analysis of spatial variation in house sparrow CORTf, and from another examination of spatial CORTp variation (Busch *et al.* 2011), is that precipitation is a dominant factor influencing broad-scale variation in CORT levels in space. To further investigate this hypothesis more large-scale studies investigating CORT variation and relating these patterns to weather conditions are needed. Both studies on which this hypothesis is

based were conducted in relatively tropical areas (Mexico and Panama), and it remains to be determined whether this association holds in more temperate regions. Another interesting direction would be to use captive birds to assess the effects of restricted access to water on CORTf levels, as elevated cortisol levels have been found in horses deprived of access to water compared to controls (Friend 2000).

Analysis of temporal variation in CORTf of tree sparrows in Illinois indicated that in the mid-eastern U. S. the early 1980s were an unusually energetically demanding period for birds compared to other years from the mid-1960s to the late 1980s. To determine if this pattern is seen in other species this study could be repeated using feather collections or museum specimens sampled over a similar time period and preferably from a similar area. It may be rare to find collections meeting these criteria, so another approach is to identify more recent periods where conditions were similar to those in Illinois in the 1980s (severe El Niño event, low corn yields due to moisture stress, etc.) and determine whether these periods were associated with unusually high CORTf levels. This type of analysis would help isolate which environmental factors were likely responsible for the 1980s CORTf increase in tree sparrows.

All future studies of temporal CORT variation are dependent on access to CORT data collected over long time periods. To make this work possible in the future, established, long-term study sites should consider collecting feathers each year so that temporal changes in CORTf in their study population can be tracked. Especially for species for which there are few museum specimens to use as a reference point for historical variation in CORTf this information is critical for determining whether future CORTf increases in response to environmental change are cause for concern or if they fall within the normal range of variation.

This study was the first to explore CORT-weather relationships using feather CORT. Several previous studies have explored associations between CORTp or CORTfe and weather in different species and contexts, but more work is needed. Studies incorporating multiple measures of CORT can help clarify how using blood, fecal, or feather CORT measures may affect interpretation of these relationships. This study suggested that weather conditions in months prior to feather growth influence CORTf and also that the direction of CORTf-precipitation relationships is not always consistent over the course of the annual cycle. These hypotheses should be further explored with more studies relating CORTf levels to weather conditions measured over multiple time scales. It is important to note that the time scale over which weather is considered must be appropriate to the measure of CORT used. Monthly measures were relevant for this study because CORTf incorporates CORT secretion over a period of weeks. Bize *et al*. (2010) measured weather conditions for various periods in the 42 hours prior to blood sampling, which is more relevant to this instantaneous measure of CORT secretion. As CORTf incorporates the amplitude and duration of all CORT elevations occurring during the period of feather growth (Bortolotti *et al*. 2008) the CORTf value may be strongly influenced by the number of 'peaks', or periods of elevated CORT secretion. In this case, mean monthly temperature may not be strongly associated with CORTf compared to the number of days in a month with extreme high or low temperatures or levels of precipitation. Rather than using monthly measures it would be ideal to relate CORTf to weather conditions during the exact period of feather growth; however it would be difficult to determine exactly when this occurs for individuals in the wild. One way to get around this is to pull a feather to induce growth of a replacement feather then relate the amount of CORT in the replacement feather to weather conditions during the exact time period over which it was grown. An experiment like this could

have another treatment group in which CORTp levels were related to short-term weather conditions at the same time, to determine whether the CORT measure used affects conclusions regarding CORT-weather relationships.

My results are novel because they are the first application of feather CORT to a macrophysiological context. It is clear that this measure can reveal how CORT varies across broad spatial and temporal scales. Information about broad-scale variation in a physiological measure that is tied to energetic requirements is highly relevant for conservation. Due to the ease of feather sampling this method should be used to provide these data for other species. My results are also the first to demonstrate links between CORTf and weather variables. Although similar relationships have been shown using CORTp and CORTfe, feather CORT is unique in that it can be used retrospectively. As shown in the tree sparrow study, CORTf can tell us how birds were coping with past environmental conditions. Knowledge of how birds respond to a range of weather conditions can allow us to predict how populations will respond to future changes. This critical information can help conservationists put measures in place pre-emptively, potentially mitigating or even avoiding population declines and extinctions.

These data also add to a growing body of evidence showing that feather CORT provides valuable information about how birds cope with environmental challenges (Bortolotti *et al*. 2009a; Harms *et al*. 2010; Fairhurst *et al*. 2011; Fairhurst 2011; Fairhurst *et al*. 2012a; Fairhurst *et al*. 2012b). Many avian species are currently listed as threatened or endangered due to natural and anthropogenic factors including climate change and habitat loss or degradation (Both *et al*. 2006; NABCI Canada 2012; Eglington *et al*. 2012). It is highly desirable to expand the 'toolbox' available to biologists for determining how to manage habitats and other factors such as predator and prey populations in order to establish stable or increasing bird populations. While breeding

indices have traditionally been used as measures of habitat quality, this information does not help identify ideal conditions during other stages of the annual cycle. Recent migratory bird research has emphasized the need for expanding our knowledge of how conditions during wintering and migration affect populations (Rappole and McDonald 1994; Webster *et al*. 2002; Norris *et al*. 2004), and the same could be said for non-migratory species. The data presented here show that feather CORT is a powerful tool for assessing how habitat conditions are translated into energetic costs or benefits for birds. This measure can therefore help fill this knowledge gap and ultimately allow us to more effectively conserve all bird species.

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Appendix 1: Feather CORT Data

Table A1.1: Feather CORT data for 449 house sparrows sampled from 49 sites actoss Mexico from Dec. 2006 – March 2007. The 'SampleID' column lists band numbers and the 'LabID' column lists identification numbers used in a previous analysis of feather deuterium of these individuals. Feathers were processed to extract CORT in 2011, feather CORT values are listed in pg/mm. Age classes are: hatch year, HY; after hatch year, AHY; second year, SY; after second year, ASY; and unknown, U.

SampleID	LabID	Feather CORT	Age	Sex	Site	Latitude	Longitude
1451-76503	G-50203	5.97	SY	M	LACO	18.71375	-97.6729
1451-76504	G-50204	5.86	SY	M	LACO	18.71375	-97.6729
1451-76505	G-50205	5.52	SY	M	LACO	18.71375	-97.6729
1451-76506	G-50206	6.03	SY	M	LACO	18.71375	-97.6729
1451-76507	G-50207	5.60	SY	M	LACO	18.71375	-97.6729
1451-76508	G-50208	5.30	SY	M	LACO	18.71375	-97.6729
1451-76509	G-50209	5.32	SY	M	LACO	18.71375	-97.6729
1451-76510	G-50345	6.45	SY	M	MATE	16.96962	-97.9122
1451-76511	G-50346	4.96	AHY	F	MATE	16.96962	-97.9122
1451-76512	G-50347	4.63	AHY	F	MATE	16.96962	-97.9122
1451-76513	G-50348	4.61	AHY	F	MATE	16.96962	-97.9122
1451-76514	G-50349	4.41	ASY	M	MATE	16.96962	-97.9122
1451-76515	G-50350	4.35	ASY	M	MATE	16.96962	-97.9122
1451-76516	G-50210	6.20	AHY	F	SAN1	16.77661	-99.4259
1451-76517	G-50211	5.87	AHY	F	SAN1	16.77661	-99.4259
1451-76519	G-50352	5.80	SY	F	TEC1	17.235	-100.631
1451-76520	G-50353	4.68	SY	F	TEC1	17.235	-100.631
1451-76521	G-50354	6.27	AHY	F	TEC1	17.235	-100.631
1451-76523	G-50356	4.38	AHY	F	TEC1	17.235	-100.631
1451-76524	G-50357	5.15	SY	M	TEC1	17.235	-100.631
1451-76525	G-50358	5.71	SY	M	TEC1	17.235	-100.631
1451-76526	G-50359	5.73	AHY	F	TEC1	17.235	-100.631
1451-76527	G-50360	5.32	SY	M	TEC1	17.235	-100.631
1451-76528	G-50361	6.49	ASY	M	TEC1	17.235	-100.631
1451-76529	G-53263	3.20	ASY	M	MOLO	19.05987	-104.258
1451-76531	G-53261	5.87	ASY	M	MAR1	20.95261	-105.33
1451-76532	G-53260	4.92	AHY	F	MAR1	20.95261	-105.33
1451-76533	G-53259	6.12	AHY	F	MAR1	20.95261	-105.33
1451-76535	G-53257	4.25	AHY	F	MAR1	20.95261	-105.33
1451-76536	G-53256	4.18	AHY	F	MAR1	20.95261	-105.33
1451-76537	G-53255	3.54	ASY	M	MAR1	20.95261	-105.33
1451-76538	G-53254	3.71	ASY	M	MAR1	20.95261	-105.33
1451-76539	G-53253	4.00	AHY	F	MAR1	20.95261	-105.33
1451-76540	G-50212	5.29	ASY	M	ZAP2	20.46668	-102.931

1451-76541	G-50213	5.57	ASY	M	ZAP2	20.46668	-102.931
1451-76542	G-50214	5.95	AHY	M	ZAP2	20.46668	-102.931
1451-76543	G-50215	3.36	AHY	M	ZAP2	20.46668	-102.931
1451-76545	G-50217	6.51	AHY	M	ZAP2	20.46668	-102.931
1451-76546	G-50218	5.21	AHY	F	ZAP2	20.46668	-102.931
1451-76547	G-50219	6.16	SY	M	ZAP2	20.46668	-102.931
1451-76548	G-50220	5.16	AHY	M	ZAP2	20.46668	-102.931
1451-76549	G-50221	4.63	SY	M	ZAP2	20.46668	-102.931
1451-76550	G-50222	4.60	AHY	F	ZAP2	20.46668	-102.931
1451-76551	G-50223	5.33	SY	M	ZAP2	20.46668	-102.931
1451-76553	G-50225	6.01	SY	M	QUIR	19.65742	-101.529
1451-76554	G-50226	4.75	SY	M	QUIR	19.65742	-101.529
1451-76555	G-50227	6.30	AHY	M	QUIR	19.65742	-101.529
1451-76556	G-50228	4.54	AHY	M	QUIR	19.65742	-101.529
1451-76557	G-50229	5.86	AHY	M	QUIR	19.65742	-101.529
1451-76558	G-50230	3.86	AHY	F	QUIR	19.65742	-101.529
1451-76559	G-50231	4.90	AHY	F	QUIR	19.65742	-101.529
1451-76560	G-50232	4.14	AHY	M	QUIR	19.65742	-101.529
1451-76561	G-50233	4.39	SY	M	QUIR	19.65742	-101.529
1451-76562	G-50234	3.27	SY	M	QUIR	19.65742	-101.529
1451-76563	G-50235	3.31	SY	M	QUIR	19.65742	-101.529
1451-76564	G-50391	4.12	SY	M	PRES	20.87935	-100.777
1451-76565	G-50392	5.39	AHY	F	PRES	20.87935	-100.777
1451-76566	G-50393	3.60	AHY	F	PRES	20.87935	-100.777
1451-76567	G-50394	3.87	AHY	F	PRES	20.87935	-100.777
1451-76568	G-50395	3.97	ASY	M	PRES	20.87935	-100.777
1451-76569	G-50396	4.24	AHY	M	PRES	20.87935	-100.777
1451-76570	G-50397	3.33	ASY	M	PRES	20.87935	-100.777
1451-76571	G-50398	5.14	SY	M	PRES	20.87935	-100.777
1451-76572	G-50252	6.10	AHY	F	MARR	21.7231	-100.97
1451-76573	G-50253	7.01	AHY	M	MARR	21.7231	-100.97
1451-76574	G-50254	2.69	AHY	F	MARR	21.7231	-100.97
1451-76577	G-50257	2.74	SY	M	MARR	21.7231	-100.97
1451-76578	G-50258	8.68	SY	M	MARR	21.7231	-100.97
1451-76579	G-50259	10.35	SY	M	MARR	21.7231	-100.97
1451-76581	G-50260	6.49	SY	M	MARR	21.7231	-100.97
1451-76582	G-50261	3.80	AHY	M	MARR	21.7231	-100.97
1451-76583	G-50262	4.26	SY	M	MARR	21.7231	-100.97
1451-76584	G-50263	3.47	SY	M	MARR	21.7231	-100.97
1451-76585	G-50264	6.27	AHY	F	MARR	21.7231	-100.97
1451-76586	G-50265	5.39	AHY	F	SNIL	23.15911	-102.851
1451-76587	G-50266	6.08	AHY	F	SNIL	23.15911	-102.851
1451-76588	G-50267	3.98	SY	M	SNIL	23.15911	-102.851
1451-76589	G-50268	4.59	AHY	F	SNIL	23.15911	-102.851
1451-76590	G-50269	6.74	AHY	F	SNIL	23.15911	-102.851

1451-76591	G-50270	4.66	ASY	M	SNIL	23.15911	-102.851
1451-76592	G-53264	4.42	AHY	M	RANG	24.07586	-104.656
1451-76593	G-53162	4.09	AHY	F	NATO	24.61479	-104.642
1451-76595	G-53164	4.95	AHY	F	NATO	24.61479	-104.642
1451-76597	G-50273	3.49	SY	M	TONI	25.12865	-104.537
1451-76598	G-50271	6.33	SY	M	TONI	25.12865	-104.537
1451-76599	G-50272	3.88	AHY	F	TONI	25.12865	-104.537
1451-76600	G-50274	2.55	AHY	F	TONI	25.12865	-104.537
1451-76701	G-50275	3.25	SY	M	TONI	25.12865	-104.537
1451-76702	G-50276	4.46	SY	M	TONI	25.12865	-104.537
1451-76703	G-50277	5.91	SY	M	TONI	25.12865	-104.537
1451-76704	G-50278	4.53	AHY	M	TONI	25.12865	-104.537
1451-76705	G-50279	4.54	AHY	F	TONI	25.12865	-104.537
1451-76706	G-50280	5.03	AHY	M	TONI	25.12865	-104.537
1451-76707	G-50281	3.56	AHY	M	TONI	25.12865	-104.537
1451-76708	G-50282	4.32	AHY	F	TONI	25.12865	-104.537
1451-76709	G-50283	6.35	ASY	M	TONI	25.12865	-104.537
1451-76710	G-50284	5.44	AHY	F	TONI	25.12865	-104.537
1451-76711	G-50285	5.64	AHY	M	TONI	25.12865	-104.537
1451-76712	G-50286	4.40	AHY	F	TONI	25.12865	-104.537
1451-76713	G-50287	4.85	AHY	F	TONI	25.12865	-104.537
1451-76714	G-50372	5.92	AHY	M	SPER	25.86036	-104.822
1451-76715	G-50371	5.01	ASY	M	SPER	25.86036	-104.822
1451-76716	G-50364	5.63	AHY	M	SPER	25.86036	-104.822
1451-76717	G-50365	6.05	ASY	M	SPER	25.86036	-104.822
1451-76718	G-50366	4.30	AHY	M	SPER	25.86036	-104.822
1451-76719	G-50367	5.45	AHY	F	SPER	25.86036	-104.822
1451-76720	G-50368	5.55	ASY	M	SPER	25.86036	-104.822
1451-76721	G-50369	6.30	AHY	F	SPER	25.86036	-104.822
1451-76722	G-50370	5.87	SY	M	SPER	25.86036	-104.822
1451-76723	G-50362	9.06	SY	M	SPER	25.86036	-104.822
1451-76724	G-50363	6.15	AHY	F	SPER	25.86036	-104.822
1451-76725	G-50373	5.90	SY	M	ZARA	27.45398	-105.819
1451-76726	G-50374	7.87	AHY	M	ZARA	27.45398	-105.819
1451-76727	G-50375	9.59	ASY	M	ZARA	27.45398	-105.819
1451-76728	G-50376	8.52	AHY	F	ZARA	27.45398	-105.819
1451-76729	G-50377	19.19	AHY	M	ZARA	27.45398	-105.819
1451-76730	G-50378	32.36	ASY	M	ZARA	27.45398	-105.819
1451-76731	G-50379	18.32	SY	M	ZARA	27.45398	-105.819
1451-76732	G-50288	14.49	AHY	F	RICA	29.95166	-106.963
1451-76733	G-50289	5.48	ASY	M	RICA	29.95166	-106.963
1451-76734	G-50290	12.85	AHY	F	RICA	29.95166	-106.963
1451-76735	G-50291	12.56	AHY	F	RICA	29.95166	-106.963
1451-76736	G-50292	10.26	ASY	M	RICA	29.95166	-106.963
1451-76737	G-50293	9.96	AHY	M	RICA	29.95166	-106.963

1451-76738	G-50294	17.43	SY	M	RICA	29.95166	-106.963
1451-76739	G-50295	6.67	AHY	F	RICA	29.95166	-106.963
1451-76740	G-50296	6.36	SY	M	RICA	29.95166	-106.963
1451-76741	G-50297	11.54	AHY	M	RICA	29.95166	-106.963
1451-76742	G-50298	11.91	AHY	F	RICA	29.95166	-106.963
1451-76743	G-50299	11.96	AHY	F	RICA	29.95166	-106.963
1451-76744	G-50300	13.89	ASY	M	RICA	29.95166	-106.963
1451-76745	G-50301	10.05	AHY	F	RICA	29.95166	-106.963
1451-76746	G-50302	6.38	ASY	M	RICA	29.95166	-106.963
1451-76747	G-50303	16.44	AHY	F	RICA	29.95166	-106.963
1451-76748	G-50380	6.98	AHY	F	SACR	28.85377	-106.203
1451-76749	G-50381	13.67	AHY	F	SACR	28.85377	-106.203
1451-76750	G-50382	6.81	AHY	F	SACR	28.85377	-106.203
1451-76751	G-50383	5.36	AHY	M	SACR	28.85377	-106.203
1451-76752	G-50384	7.18	AHY	M	SACR	28.85377	-106.203
1451-76753	G-50385	4.36	AHY	F	SACR	28.85377	-106.203
1451-76754	G-50386	4.37	AHY	F	SACR	28.85377	-106.203
1451-76755	G-50387	10.45	SY	M	SACR	28.85377	-106.203
1451-76756	G-50388	5.87	AHY	F	SACR	28.85377	-106.203
1451-76757	G-50389	5.29	AHY	F	SACR	28.85377	-106.203
1451-76758	G-50390	18.38	SY	M	SACR	28.85377	-106.203
1451-76760	G-50237	4.10	AHY	F	VENT	25.83524	-103.601
1451-76761	G-50238	4.36	ASY	M	VENT	25.83524	-103.601
1451-76762	G-50239	4.59	AHY	M	VENT	25.83524	-103.601
1451-76763	G-50240	5.11	ASY	M	VENT	25.83524	-103.601
1451-76764	G-50241	5.11	AHY	F	VENT	25.83524	-103.601
1451-76765	G-50242	3.95	SY	M	VENT	25.83524	-103.601
1451-76767	G-50244	5.43	SY	M	VENT	25.83524	-103.601
1451-76768	G-50245	4.38	AHY	F	VENT	25.83524	-103.601
1451-76769	G-50246	4.10	AHY	M	VENT	25.83524	-103.601
1451-76770	G-50247	5.29	ASY	M	VENT	25.83524	-103.601
1451-76771	G-50248	5.29	ASY	M	VENT	25.83524	-103.601
1451-76772	G-50249	7.96	AHY	F	VENT	25.83524	-103.601
1451-76773	G-50250	8.02	AHY	M	VENT	25.83524	-103.601
1451-76774	G-50251	5.00	ASY	M	VENT	25.83524	-103.601
1451-76775	G-50399	5.98	AHY	M	SAC1	26.99818	-101.72
1451-76776	G-50400	6.63	SY	M	SAC1	26.99818	-101.72
1451-76777	G-50401	6.38	AHY	M	SAC1	26.99818	-101.72
1451-76778	G-50402	4.64	AHY	M	SAC1	26.99818	-101.72
1451-76779	G-50403	4.47	AHY	F	SAC1	26.99818	-101.72
1451-76780	G-50404	6.23	ASY	M	SAC1	26.99818	-101.72
1451-76781	G-50405	9.35	ASY	M	SAC1	26.99818	-101.72
1451-76782	G-50406	5.17	AHY	F	SAC1	26.99818	-101.72
1451-76783	G-50407	6.07	SY	M	SAC1	26.99818	-101.72
1451-76784	G-50408	11.58	ASY	M	SAC1	26.99818	-101.72

1451-76785	G-50409	7.37	AHY	F	SAC1	26.99818	-101.72
1451-76786	G-50410	7.57	AHY	F	SAC1	26.99818	-101.72
1451-76787	G-50411	6.14	ASY	M	SAC1	26.99818	-101.72
1451-76788	G-50412	5.80	AHY	F	SAC1	26.99818	-101.72
1451-76789	G-50413	4.69	AHY	M	SAC1	26.99818	-101.72
1451-76790	G-50414	6.93	AHY	M	SAC1	26.99818	-101.72
1451-76791	G-50415	8.09	AHY	M	SAC1	26.99818	-101.72
1451-76792	G-50416	7.05	AHY	M	SAC1	26.99818	-101.72
1451-76793	G-50417	4.84	AHY	M	TANO	26.75202	-101.41
1451-76794	G-50418	7.30	AHY	F	TANO	26.75202	-101.41
1451-76795	G-50419	6.85	AHY	F	TANO	26.75202	-101.41
1451-76796	G-50420	3.40	AHY	F	TANO	26.75202	-101.41
1451-76797	G-50421	6.39	ASY	M	TANO	26.75202	-101.41
1451-76798	G-50422	4.58	AHY	F	TANO	26.75202	-101.41
1451-76799	G-50423	4.70	SY	M	TANO	26.75202	-101.41
1451-76800	G-50424	5.61	AHY	F	TANO	26.75202	-101.41
1451-90001	G-50304	4.68	AHY	F	CRBL	19.63744	-97.1633
1451-90002		5.99			CRBL		
1451-90003	G-50306	4.98	AHY	M	CRBL	19.63744	-97.1633
1451-90004	G-50307	4.64	SY	M	CRBL	19.63744	-97.1633
1451-90005	G-50308	5.01	SY	M	CRBL	19.63744	-97.1633
1451-90006	G-50309	5.46	SY	F	CRBL	19.63744	-97.1633
1451-90008	G-50310	4.72	ASY	M	LACO	18.71375	-97.6729
1451-90009	G-50311	5.30	SY	M	LACO	18.71375	-97.6729
1451-90012	G-50314	4.32	AHY	F	CARC	16.33886	-92.5653
1451-90013	G-50315	3.98	AHY	F	CARC	16.33886	-92.5653
1451-90014	G-50316	5.06	AHY	M	CARC	16.33886	-92.5653
1451-90015	G-50317	6.90	AHY	F	CARC	16.33886	-92.5653
1451-90016	G-50318	3.80	AHY	F	CARC	16.33886	-92.5653
1451-90017	G-50319	3.96	AHY	F	CARC	16.33886	-92.5653
1451-90018	G-53172	4.76	AHY	F	B001	23.45581	-105.828
1451-90019	G-53173	5.72	AHY	M	B001	23.45581	-105.828
1451-90020	G-53174	3.53	AHY	F	B001	23.45581	-105.828
1451-90021	G-53175	4.33	AHY	F	B001	23.45581	-105.828
1451-90022	G-53176	3.99	AHY	F	B001	23.45581	-105.828
1451-90023	G-53177	3.21	ASY	M	B001	23.45581	-105.828
1451-90024	G-53178	4.59	AHY	F	B001	23.45581	-105.828
1451-90025	G-53179	4.05	AHY	F	B001	23.45581	-105.828
1451-90026	G-53180	4.36	AHY	F	B001	23.45581	-105.828
1451-90027	G-53181	3.23	AHY	F	B001	23.45581	-105.828
1451-90028	G-53223	4.41	AHY	F	B006	24.17617	-107.102
1451-90029	G-53224	4.49	AHY	F	B006	24.17617	-107.102
1451-90030	G-53225	2.88	AHY	F	B006	24.17617	-107.102
1451-90031	G-53226	4.68	AHY	F	B006	24.17617	-107.102
1451-90032	G-53227	3.67	AHY	F	B006	24.17617	-107.102

1451-90033	G-53228	2.80	AHY	F	B006	24.17617	-107.102
1451-90034	G-53229	5.36	ASY	M	B006	24.17617	-107.102
1451-90035	G-53230	2.98	ASY	M	B006	24.17617	-107.102
1451-90036	G-53231	5.78	ASY	M	B006	24.17617	-107.102
1451-90037	G-53232	3.45	AHY	F	B006	24.17617	-107.102
1451-90038	G-53233	5.18	SY	M	B006	24.17617	-107.102
1451-90039	G-53182	3.51	ASY	M	B012	25.88251	-109.009
1451-90040	G-53183	4.27	ASY	M	B012	25.88251	-109.009
1451-90041	G-53184	5.55	AHY	F	B012	25.88251	-109.009
1451-90042	G-53185	3.74	AHY	F	B012	25.88251	-109.009
1451-90043	G-53186	3.24	ASY	M	B012	25.88251	-109.009
1451-90044	G-53187	4.29	ASY	M	B012	25.88251	-109.009
1451-90045	G-53188	4.05	AHY	M	B012	25.88251	-109.009
1451-90046	G-53189	3.90	AHY	F	B012	25.88251	-109.009
1451-90047	G-53190	4.22	ASY	M	B012	25.88251	-109.009
1451-90048	G-53191	4.04	AHY	M	B012	25.88251	-109.009
1451-90049	G-53222	4.90	ASY	M	B012	25.88251	-109.009
1451-90049	G-53192	5.74	ASY	M	B012	25.88251	-109.009
1451-90050	G-53193	4.42	AHY	F	B012	25.88251	-109.009
1451-90051	G-53194	5.58	AHY	M	B012	25.88251	-109.009
1451-90052	G-53195	4.17	ASY	M	B012	25.88251	-109.009
1451-90053	G-53196	3.72	AHY	M	B012	25.88251	-109.009
1451-90054	G-53197	3.56	AHY	F	B012	25.88251	-109.009
1451-90055	G-53234	4.99	AHY	F	B015	27.33023	-109.733
1451-90056	G-53235	4.49	AHY	M	B015	27.33023	-109.733
1451-90057	G-53236	4.40	ASY	M	B015	27.33023	-109.733
1451-90058	G-53237	4.79	AHY	M	B015	27.33023	-109.733
1451-90059	G-53238	4.83	ASY	M	B015	27.33023	-109.733
1451-90060	G-53239	6.11	AHY	M	B015	27.33023	-109.733
1451-90061	G-53240	4.92	AHY	M	B015	27.33023	-109.733
1451-90062	G-53241	8.92	AHY	F	B015	27.33023	-109.733
1451-90063	G-53242	5.30	AHY	F	B015	27.33023	-109.733
1451-90064	G-53243	3.89	AHY	M	B015	27.33023	-109.733
1451-90065	G-53244	5.06	ASY	M	B015	27.33023	-109.733
1451-90066	G-53245	4.61	ASY	M	B015	27.33023	-109.733
1451-90068	G-53198	5.24	ASY	M	B018	27.93818	-110.936
1451-90069	G-53199	5.83	AHY	M	B018	27.93818	-110.936
1451-90070	G-53200	4.88	AHY	F	B018	27.93818	-110.936
1451-90071	G-53201	4.81	ASY	M	B018	27.93818	-110.936
1451-90072	G-53202	4.47	ASY	M	B018	27.93818	-110.936
1451-90073	G-53203	3.66	ASY	M	B018	27.93818	-110.936
1451-90074	G-53246	6.33	ASY	M	B024	28.56873	-109.579
1451-90075	G-53247	4.44	AHY	M	B024	28.56873	-109.579
1451-90076	G-53248	4.90	AHY	F	B024	28.56873	-109.579
1451-90077	G-53249	6.19	AHY	F	B024	28.56873	-109.579

1451-90078	G-53250	5.40	AHY	F	B024	28.56873	-109.579
1451-90079	G-53251	6.51	AHY	M	B024	28.56873	-109.579
1451-90080	G-53252	4.97	AHY	F	B024	28.56873	-109.579
1451-90081	G-53204	4.78	AHY	F	B031	28.21689	-108.225
1451-90082	G-53205	6.33	AHY	F	B031	28.21689	-108.225
1451-90083	G-53206	6.93	AHY	F	B031	28.21689	-108.225
1451-90084	G-53207	5.84	AHY	F	B031	28.21689	-108.225
1451-90085	G-53208	5.71	AHY	F	B031	28.21689	-108.225
1451-90086	G-53209	3.99	AHY	F	B031	28.21689	-108.225
1451-90087	G-53210	4.92	AHY	F	B031	28.21689	-108.225
1451-90088	G-53211	6.34	ASY	M	B031	28.21689	-108.225
1451-90089	G-53212	6.05	AHY	F	B031	28.21689	-108.225
1451-90090	G-53213	6.72	AHY	M	B031	28.21689	-108.225
1451-90091	G-53214	4.82	ASY	M	B031	28.21689	-108.225
1451-90094	G-53215	6.06	AHY	F	B038	30.61046	-106.511
1451-90095	G-53216	4.95	AHY	M	B038	30.61046	-106.511
1451-90096	G-53217	7.43	ASY	M	B038	30.61046	-106.511
1451-90097	G-53218	6.70	ASY	M	B038	30.61046	-106.511
1451-90098	G-53219	6.96	ASY	M	B038	30.61046	-106.511
1451-90099	G-53220	4.98	AHY	F	B038	30.61046	-106.511
1451-90100	G-53221	7.42	ASY	M	B038	30.61046	-106.511
1451-90401	G-50425	7.15	AHY	F	TANO	26.75202	-101.41
1451-90402	G-50426	6.21	ASY	M	TANO	26.75202	-101.41
1451-90403	G-50427	4.78	ASY	M	TADA	25.28699	-101.085
1451-90404	G-50428	4.12	ASY	M	TADA	25.28699	-101.085
1451-90405	G-50429	4.45	AHY	M	TADA	25.28699	-101.085
1451-90406	G-50430	5.62	ASY	M	TADA	25.28699	-101.085
1451-90407	G-50431	4.59	ASY	M	TADA	25.28699	-101.085
1451-90408	G-50320	5.53	AHY	F	RANC	23.42107	-98.9789
1451-90409	G-50321	4.35	AHY	F	RANC	23.42107	-98.9789
1451-90410	G-50322	5.88	AHY	F	RANC	23.42107	-98.9789
1451-90411	G-50323	5.00	AHY	F	RANC	23.42107	-98.9789
1451-90412	G-50324	4.05	AHY	F	RANC	23.42107	-98.9789
1451-90413	G-50325	6.76	AHY	F	RANC	23.42107	-98.9789
1451-90414	G-50326	5.96	ASY	F	RANC	23.42107	-98.9789
1451-90415	G-50327	6.08	AHY	F	RANC	23.42107	-98.9789
1451-90416	G-50432	5.63	AHY	M	RMAS	21.69752	-98.9676
1451-90417	G-50433	6.09	AHY	F	RMAS	21.69752	-98.9676
1451-90418	G-50434	4.09	ASY	M	RMAS	21.69752	-98.9676
1451-90419	G-50435	5.77	AHY	F	RMAS	21.69752	-98.9676
1451-90420	G-50436	8.31	AHY	F	RMAS	21.69752	-98.9676
1451-90421	G-50437	5.13	ASY	F	RMAS	21.69752	-98.9676
1451-90422	G-50438	7.28	AHY	F	RMAS	21.69752	-98.9676
1451-90423	G-50439	5.76	ASY	M	RMAS	21.69752	-98.9676
1451-90424	G-50440	5.50	AHY	M	RMAS	21.69752	-98.9676

1451-90425	G-50441	5.27	AHY	F	RMAS	21.69752	-98.9676
1451-90426	G-50328	6.52	AHY	F	REME	20.53033	-99.3173
1451-90427	G-50329	5.69	AHY	F	REME	20.53033	-99.3173
1451-90428	G-50330	4.57	AHY	M	REME	20.53033	-99.3173
1451-90429	G-50331	3.37	SY	M	REME	20.53033	-99.3173
1451-90430	G-50332	3.79	AHY	F	REME	20.53033	-99.3173
1451-90431	G-50333	2.50	AHY	F	REME	20.53033	-99.3173
1451-90433	G-50335	2.71	ASY	M	REME	20.53033	-99.3173
1451-90434	G-50336	6.34	AHY	F	REME	20.53033	-99.3173
1451-90435	G-50337	6.27	ASY	M	REME	20.53033	-99.3173
1451-90436	G-50338	6.29	AHY	F	REME	20.53033	-99.3173
1451-90437	G-50339	3.55	AHY	M	REME	20.53033	-99.3173
1451-90438	G-50340	5.33	AHY	F	REME	20.53033	-99.3173
1451-90439	G-50341	2.72	ASY	M	REME	20.53033	-99.3173
1451-90440	G-50342	3.10	AHY	F	REME	20.53033	-99.3173
1451-90441	G-50343	5.99	AHY	F	REME	20.53033	-99.3173
1451-90442	G-50344	3.07	AHY	F	REME	20.53033	-99.3173
1451-90443	G-50442	3.04	AHY	F	SAN3	19.93762	-98.5967
1451-90444	G-50443	5.28	AHY	F	SAN3	19.93762	-98.5967
1451-90445	G-50444	6.18	AHY	F	SAN3	19.93762	-98.5967
1451-90446	G-50445	6.13	ASY	M	SAN3	19.93762	-98.5967
1451-90447	G-50446	3.34	ASY	M	SAN3	19.93762	-98.5967
1451-90448	G-50447	6.72	ASY	M	SAN3	19.93762	-98.5967
1451-90450	G-53113	5.93	ASY	M	B043	31.09423	-107.989
1451-90451	G-53114	5.58	ASY	M	B043	31.09423	-107.989
1451-90452	G-53115	4.39	ASY	M	B043	31.09423	-107.989
1451-90453	G-53116	7.07	ASY	M	B043	31.09423	-107.989
1451-90454	G-53117	3.85	ASY	M	B043	31.09423	-107.989
1451-90455	G-53118	7.04	ASY	M	B043	31.09423	-107.989
1451-90456	G-53119	9.77	ASY	M	B043	31.09423	-107.989
1451-90457	G-53120	5.40	AHY	M	B043	31.09423	-107.989
1451-90459	G-53122	6.10	AHY	M	B043	31.09423	-107.989
1451-90460	G-53123	6.58	AHY	M	B043	31.09423	-107.989
1451-90461	G-53124	6.43	AHY	F	B043	31.09423	-107.989
1451-90462	G-53125	4.02	AHY	M	B043	31.09423	-107.989
1451-90463	G-53126	6.97	AHY	M	B043	31.09423	-107.989
1451-90464	G-53127	5.77	ASY	M	B043	31.09423	-107.989
1451-90465	G-53128	7.24	AHY	M	B043	31.09423	-107.989
1451-90466	G-53129	6.87	ASY	M	B043	31.09423	-107.989
1451-90467	G-53130	4.35	ASY	M	B043	31.09423	-107.989
1451-90468	G-53131	9.29	ASY	M	B043	31.09423	-107.989
1451-90469	G-53132	7.04	AHY	F	B043	31.09423	-107.989
1451-90470	G-53133	4.03	ASY	M	B043	31.09423	-107.989
1451-90471	G-53134	3.45	AHY	F	B047	31.31389	-109.584
1451-90472	G-53135	3.90	AHY	F	B047	31.31389	-109.584

1451-90474	G-53137	8.22	AHY	F	B047	31.31389	-109.584
1451-90475	G-53138	7.30	ASY	M	B047	31.31389	-109.584
1451-90476	G-53139	6.87	AHY	F	B051	30.83893	-110.837
1451-90477	G-53140	6.50	AHY	F	B051	30.83893	-110.837
1451-90478	G-53141	4.43	SY	M	B051	30.83893	-110.837
1451-90479	G-53142	3.56	AHY	F	B051	30.83893	-110.837
1451-90480	G-53143	3.82	ASY	M	B051	30.83893	-110.837
1451-90481	G-53144	3.74	AHY	M	B051	30.83893	-110.837
1451-90482	G-53145	3.38	ASY	M	B051	30.83893	-110.837
1451-90483	G-53146	6.34	AHY	F	B051	30.83893	-110.837
1451-90484	G-53147	4.43	ASY	M	B051	30.83893	-110.837
1451-90485	G-53148	6.48	SY	M	B051	30.83893	-110.837
1451-90486	G-53149	3.69	AHY	F	B051	30.83893	-110.837
1451-90487	G-53150	7.08	AHY	F	B055	29.17527	-110.895
1451-90488	G-53151	3.81	ASY	M	B055	29.17527	-110.895
1451-90489	G-53152	4.17	AHY	F	B055	29.17527	-110.895
1451-90490	G-53153	3.55	AHY	F	B055	29.17527	-110.895
1451-90491	G-53154	5.63	ASY	M	B055	29.17527	-110.895
1451-90492	G-53155	3.76	ASY	M	B055	29.17527	-110.895
1451-90493	G-53156	8.54	AHY	F	B055	29.17527	-110.895
1451-90494	G-53157	6.64	AHY	F	B055	29.17527	-110.895
1451-90495	G-53158	6.09	ASY	M	B055	29.17527	-110.895
1451-90496	G-53159	5.85	ASY	M	B055	29.17527	-110.895
1451-90497	G-53160	8.27	ASY	M	B055	29.17527	-110.895
1451-90498	G-53161	5.95	ASY	M	B055	29.17527	-110.895
2241-67601	G-57565	6.88	HY	M	D7-TOSA	23.44228	-110.227
2241-67602	G-57566	6.67	U	F	D7-TOSA	23.44228	-110.227
2241-67603	G-57567	7.89	HY	M	D7-TOSA	23.44228	-110.227
2241-67604	G-57568	7.91	U	F	D7-TOSA	23.44228	-110.227
2241-67605	G-57569	8.70	U	F	D7-TOSA	23.44228	-110.227
2241-67606	G-57570	6.85	U	F	D7-TOSA	23.44228	-110.227
2241-67607	G-57571	6.66	AHY	M	D7-TOSA	23.44228	-110.227
2241-67608	G-57572	8.11	AHY	M	D7-TOSA	23.44228	-110.227
2241-67609	G-57573	5.35	U	F	D7-TOSA	23.44228	-110.227
2241-67610	G-57574	6.07	AHY	M	D7-TOSA	23.44228	-110.227
2241-67611	G-57575	6.39	U	F	D7-TOSA	23.44228	-110.227
2241-67612	G-57576	7.14	U	F	D7-TOSA	23.44228	-110.227
2241-67613	G-57577	5.19	U	F	D7-TOSA	23.44228	-110.227
2241-67614	G-57578	7.45	U	M	D7-TOSA	23.44228	-110.227
2241-67615	G-57579	7.55	HY	M	D7-TOSA	23.44228	-110.227
2241-67618	G-57580	6.38	U	M	D7-SAAG	24.16076	-110.922
2241-67619	G-57581	6.62	AHY	M	D7-SAAG	24.16076	-110.922
2241-67620	G-57582	6.54	U	F	D7-SAAG	24.16076	-110.922
2241-67621	G-57583	6.69	U	F	D7-SAAG	24.16076	-110.922
2241-67622	G-57584	5.76	AHY	M	D7-SAAG	24.16076	-110.922

2241-67623	G-57585	6.24	AHY	M	D7-SAAG	24.16076	-110.922
2241-67624	G-57586	7.12	AHY	M	D7-SAAG	24.16076	-110.922
2241-67625	G-57587	6.97	U	F	D7-SAAG	24.16076	-110.922
2241-67626	G-57588	5.65	HY	M	D7-SAAG	24.16076	-110.922
2241-67627	G-57589	5.30	U	F	D7-SAAG	24.16076	-110.922
2241-67628	G-57590	8.17	U	F	D7-SAAG	24.16076	-110.922
2241-67629	G-57591	7.34	U	F	D7-CUIN	25.39863	-111.839
2241-67630	G-57592	7.26	U	M	D7-CUIN	25.39863	-111.839
2241-67631	G-57593	6.79	AHY	M	D7-CUIN	25.39863	-111.839
2241-67632		3.17	AHY	M	D7-CUIN	25.39863	-111.839
2241-67633	G-57594	3.31	HY	M	D7-GUNE	27.96787	-114.036
2241-67634	G-57595	3.69	U	M	D7-GUNE	27.96787	-114.036
2241-67635	G-57596	4.38	U	M	D7-GUNE	27.96787	-114.036
2241-67636	G-57597	3.31	U	F	D7-GUNE	27.96787	-114.036
2241-67637	G-57598	3.63	U	M	D7-GUNE	27.96787	-114.036
2241-67638	G-57599	3.73	U	F	D7-GUNE	27.96787	-114.036
2241-67639	G-57600	5.36	HY	M	D7-CATA	29.72793	-114.719
2241-67640	G-57601	3.93	U	F	D7-CATA	29.72793	-114.719
2241-67641	G-57602	6.61	U	F	D7-CATA	29.72793	-114.719
2241-67642	G-57603	3.93	AHY	M	D7-CATA	29.72793	-114.719
2241-67643	G-57604	4.14	U	F	D7-CATA	29.72793	-114.719
2241-67644	G-57605	4.02	AHY	M	D7-CATA	29.72793	-114.719
2241-67645	G-57606	5.17	U	F	D7-SAFE	30.67681	-115.978
2241-67646	G-57607	3.99	AHY	M	D7-SAFE	30.67681	-115.978
2241-67647	G-57608	4.81	U	F	D7-SAFE	30.67681	-115.978
2241-67648	G-57609	3.43	AHY	M	D7-SAFE	30.67681	-115.978
2241-67649	G-57610	4.33	U	F	D7-SAFE	30.67681	-115.978
2241-67650	G-57611	4.85	U	M	D7-SAFE	30.67681	-115.978
2241-67651	G-57612	3.76	AHY	M	D7-SAFE	30.67681	-115.978
2241-67652	G-57613	3.96	HY	M	D7-SAFE	30.67681	-115.978
2241-67653	G-57614	5.74	U	F	D7-SAAN	31.97218	-116.656
2241-67654	G-57615	5.35	U	F	D7-SAAN	31.97218	-116.656
2241-67655	G-57616	5.44	AHY	M	D7-SAAN	31.97218	-116.656
2241-67656	G-57617	5.15	U	F	D7-SAAN	31.97218	-116.656
2241-67657	G-57618	5.16	AHY	M	D7-SAAN	31.97218	-116.656
2241-67659	G-57619	3.61	HY	M	D7-SLRC	32.42539	-114.797
2241-67660	G-57620	4.90	AHY	M	D7-SLRC	32.42539	-114.797
2241-67661	G-57621	13.62	U	M	D7-SLRC	32.42539	-114.797
2241-67662	G-57622	5.59	AHY	M	D7-SLRC	32.42539	-114.797
2241-67663	G-57623	5.59	U	F	D7-SLRC	32.42539	-114.797
2241-67664	G-57624	6.15	U	F	D7-SLRC	32.42539	-114.797
2241-67665	G-57625	5.12	U	M	D7-SLRC	32.42539	-114.797
2241-67666	G-57626	5.22	U	F	D7-SLRC	32.42539	-114.797
2241-67667	G-57627	4.97	U	F	D7-SLRC	32.42539	-114.797
2241-67668	G-57628	4.94	HY	M	D7-SLRC	32.42539	-114.797

U-002	G-57629	4.05	U	F	D7-Pave	27.03026	-112.082
U-003	G-57630	4.80	AHY	M	D7-Pave	27.03026	-112.082
U-004	G-57631	5.83	HY	M	D7-Pave	27.03026	-112.082
U-005	G-57632	4.21	U	F	D7-Pave	27.03026	-112.082
U-006	G-57633	5.52	HY	M	D7-Pave	27.03026	-112.082
U-007	G-57634	4.65	HY	M	D7-Pave	27.03026	-112.082
U-008	G-57635	5.50	AHY	M	D7-Pave	27.03026	-112.082
U-009	G-57636	5.12	AHY	M	D7-Pave	27.03026	-112.082
U-010	G-57637	6.62	U	F	D7-Pave	27.03026	-112.082
U-011	G-57638	4.93	SY	M	D7-Pave	27.03026	-112.082

Table A1.2: Feather CORT data for 433 Eurasian tree sparrows sampled from 4 counties in Illinois from 1963-1990. After collection birds were prepared as study skins and stored at the Royal Ontario Museum in Toronto, ON; feathers were collected in 2009 and CORT analysis was conducted in 2011. 'ROMNO' is the specimen ID assigned by the museum. Counties are: Greene County, GC; Saint Clair County, SCC; Morgan County, MC; and Scott County, SC. All birds were assigned to one of two age classes, adult ('ad') or immature ('imm'). Body mass is listed in grams and feather CORT is listed in pg/mm.

ROMNO	County	Year	Age	Sex	Mass	Feather CORT
96441	GC	1963	ad	F	24.1	2.94
96495	GC	1963	imm	F	23.2	3.39
96465	GC	1963	ad	M	23.1	4.35
96477	GC	1963	imm	F	21.5	4.64
96483	GC	1963	ad	F	25.5	5.01
96497	GC	1963	ad	F	23	5.56
96489	GC	1963	ad	F	22.7	5.73
96485	GC	1963	ad	F	25.5	5.82
96466	GC	1963	imm	F	24.1	5.87
96479	GC	1963	imm	F	22	6.08
96468	GC	1963	imm	M	25.8	6.18
96488	GC	1963	imm	M	23.6	6.54
96480	GC	1963	ad	F	24.1	6.60
96530	SCC	1964	ad	M	23	3.99
96572	GC	1964	ad	F	21.1	4.28
96567	GC	1964	imm	F	21.2	4.42
96576	GC	1964	ad	M	24.3	4.57
96562	GC	1964	imm	M	22.7	4.81
96573	GC	1964	ad	M	24	4.88
96499	SCC	1964	ad	M	25.2	5.02
96467	GC	1964	imm	F	23	5.13
96527	SCC	1964	imm	F	21.5	5.24
96532	GC	1964	ad	M	20.9	5.30
96528	SCC	1964	imm	F	24.1	5.44
96568	GC	1964	imm	M	23.1	5.67

96487	GC	1964	ad	F	25	5.93
96575	GC	1964	ad	M	23.2	5.94
96490	GC	1964	imm	M	23.6	5.95
96478	GC	1964	imm	M	22.8	6.16
96571	GC	1964	imm	M	22.2	6.20
96526	SCC	1964	ad	F	23.1	6.21
96570	SCC	1964	ad	M	26.5	6.35
96545	SCC	1964	ad	F	22.7	6.36
96574	GC	1964	imm	F	22.2	6.41
96564	GC	1964	imm	M	22.2	6.42
96565	GC	1964	ad	F	21	6.53
96543	GC	1964	imm	M	21.6	6.56
96569	SCC	1964	ad	M	23.9	6.60
96492	GC	1964	imm	F	20.6	6.80
96577	GC	1964	imm	M	22.1	6.84
96529	SCC	1964	ad	F	24	6.89
96555	SCC	1964	imm	F	20.07	7.36
96566	GC	1964	ad	M	24.5	7.99
99064	GC	1966	.	M	23.2	3.43
99059	GC	1966	imm	F	18.1	3.85
99060	GC	1966	imm	M		4.14
97501	GC	1966	ad	M	23.3	4.38
99052	GC	1966	ad	M	24.2	4.77
99056	MC	1966	ad	F	21.6	4.90
99053	GC	1966	imm	M	23.3	4.92
99051	MC	1966	imm	M	21.3	5.46
99063	MC	1966	imm	F	21.1	5.83
99047	GC	1966	imm	F	20.7	5.90
99062	GC	1966	imm	M	20.5	5.93
99055	MC	1966	imm	F	19	6.06
99057	MC	1966	imm	F	21.6	6.25
99061	GC	1966	imm	M	23.9	6.38
99045	GC	1966	imm	M	20.9	6.61
99058	MC	1966	imm	M	20.9	8.10
99054	GC	1966	imm	M	23.4	9.81
99465	GC	1967	ad	F	23.4	6.56
100849	MC	1968	ad	M	23.3	6.81
100848	MC	1968	ad	F	22	7.78
114759	GC	1972	imm	F	23	8.03
119982	GC	1973	imm	M	22.5	4.97
119609	GC	1973	ad	M	21.3	5.01
119989	GC	1973	imm	M	22.7	5.64
119983	GC	1973	imm	F	19.5	5.79
119984	GC	1973	imm	M	21	6.19
119991	GC	1973	imm	M	21.8	6.73

119992	GC	1973	imm	F	17.5	6.90
119980	GC	1973	imm	F	18.5	8.97
119985	GC	1973	imm	F	20.1	9.34
121666	GC	1974	ad	M	21	5.10
121667	GC	1974	ad	M	22	6.00
125092	GC	1975	ad	F	24.5	7.19
125095	GC	1976	ad	M	24.7	5.67
125090	GC	1976	ad	F	21.5	6.24
125088	GC	1976	ad	F	21.5	6.25
125091	GC	1976	ad	M	22	6.36
125094	GC	1976	ad	M	23.8	6.38
125093	GC	1976	ad	M	22.5	7.02
125097	GC	1976	ad	M	23.5	7.03
125096	GC	1976	ad	F	22	7.45
125089	GC	1976	ad	F	20.3	8.75
128813	GC	1977	imm	F	22.2	3.07
128820	GC	1977	ad	M	23	5.54
128819	GC	1977	ad	M	21.4	5.62
128818	GC	1977	ad	M	22	6.00
128814	GC	1977	ad	M	22.5	6.36
128817	GC	1977	ad	M	21	7.96
128815	GC	1977	ad	M	20.5	8.31
128810	GC	1977	ad	F	23	8.55
128812	GC	1977	imm	M	25	9.23
128816	GC	1977	ad	F	20.9	9.43
128811	GC	1977	ad	M	23	9.74
132882	MC	1979	ad	M	23.1	2.32
132888	MC	1979	ad	F	21.8	3.76
132928	MC	1979	ad	F	23	3.87
132884	MC	1979	ad	F	22.5	4.27
132886	MC	1979	ad	F	22.6	4.50
132899	MC	1979	ad	M	20	4.85
132900	MC	1979	ad	F	22	5.32
132925	MC	1979	ad	M	21.7	5.96
132893	MC	1979	ad	M	21.6	6.40
132883	MC	1979	ad	F	22.7	6.56
132892	MC	1979	ad	M	22.2	6.62
133491	MC	1979	ad	F	24.9	6.66
133506	MC	1979	ad	F	23.4	6.67
133509	MC	1979	ad	F	22.4	6.68
132885	MC	1979	ad	M	22.8	6.78
133498	MC	1979	ad	F	26.7	6.95
132922	MC	1979	ad	M	21.9	7.09
132903	MC	1979	ad	M	22.8	7.17
132887	MC	1979	ad	F	21	7.31

133494	MC	1979	ad	F	23.1	7.35
132929	MC	1979	ad	F	22.5	7.35
133489	MC	1979	ad	F	26	7.38
133495	MC	1979	ad	F	25	7.41
133490	MC	1979	ad	F	20.5	7.42
132890	MC	1979	ad	F	20.5	7.53
132923	MC	1979	ad	F	21.7	7.64
132901	MC	1979	ad	F	22.2	7.86
132897	MC	1979	ad	F	23	7.95
132889	MC	1979	ad	F	23.1	8.01
133500	MC	1979	ad	F	26.5	8.10
132920	MC	1979	ad	M	22	8.26
132881	MC	1979	ad	F	22.1	8.31
132924	MC	1979	ad	M	23.6	8.44
132898	MC	1979	ad	F	22.2	8.58
132902	MC	1979	ad	M	25.3	8.66
132894	MC	1979	ad	F	21.4	8.91
132896	MC	1979	ad	M	21.5	9.06
132895	MC	1979	ad	F	23	9.14
132930	MC	1979	ad	F	21	9.55
132921	MC	1979	ad	M	23.6	9.78
133493	MC	1979	ad	F	27	10.70
137881	MC	1980	ad	F	20.5	2.49
137886	MC	1980	ad	F	22	3.35
137915	GC	1980	ad	F	21.8	4.60
137901	GC	1980	ad	F	22	4.78
137885	MC	1980	ad	F	21	4.86
137903	MC	1980	ad	M	25	5.05
137916	MC	1980	ad	F	22.5	5.07
137897	MC	1980	ad	F	22.5	5.23
137878	MC	1980	ad	M	23.5	5.85
137883	MC	1980	ad	M	26	6.02
137907	MC	1980	ad	M	18	6.11
137884	GC	1980	ad	F	25	6.57
137918	MC	1980	ad	F	22.5	6.76
137905	MC	1980	ad	M	21.5	6.86
137914	GC	1980	ad	F	24	7.05
137891	MC	1980	ad	M	23	7.13
137882	GC	1980	ad	M	22.2	7.18
137894	MC	1980	ad	F	23	7.20
137896	MC	1980	ad	F	23.5	7.25
137877	GC	1980	ad	F	25	7.37
137917	MC	1980	ad	M	23.5	7.41
137909	GC	1980	ad	M	20	7.48
137898	MC	1980	ad	F	21.5	7.54

137887	MC	1980	ad	M	22	7.66
137895	MC	1980	ad	M	22.5	8.00
137893	MC	1980	ad	M	23	8.11
137906	MC	1980	ad	F	22.5	8.37
137913	MC	1980	ad	M	23.5	8.46
137908	MC	1980	ad	M	23	8.52
137892	MC	1980	ad	M	23.5	8.59
137910	MC	1980	ad	F	23.5	8.84
137874	MC	1980	ad	M	21	8.97
137873	GC	1980	ad	M	22	9.05
137876	GC	1980	ad	M	23	9.13
137912	MC	1980	ad	M		9.24
137875	MC	1980	ad	M	24	9.51
137888	MC	1980	ad	M	21.5	9.79
137900	MC	1980	ad	F	22.5	10.82
137899	MC	1980	ad	M	22.5	11.00
137889	MC	1980	ad	F	21.5	11.29
137911	MC	1980	ad	F	22.5	11.74
137904	GC	1980	ad	M	21	15.17
141425	MC	1981	ad	M	22.5	5.26
141428	MC	1981	ad	F		5.45
141432	MC	1981	ad	M	22	5.59
141436	MC	1981	.	M	21	5.67
141426	MC	1981	ad	F	20	5.98
141433	MC	1981	ad	M	21.5	6.03
141110	MC	1981	imm	F	22	6.35
141101	MC	1981	ad	M	24	6.38
141431	MC	1981	ad	F	21	6.78
141427	MC	1981	ad	M	21	6.79
141434	MC	1981	ad	F	23.5	6.99
141112	MC	1981	ad	F	21	7.33
141429	MC	1981	ad	M	20	7.47
141116	MC	1981	ad	F	23.5	7.48
141438	MC	1981	ad	M	19.5	7.66
141424	MC	1981	ad	F	22	7.88
141435	MC	1981	ad	M	21.5	8.16
141437	MC	1981	ad	M	23	8.25
144032	MC	1982	ad	M	22	4.36
144030	MC	1982	ad	F	21	4.62
144024	MC	1982	ad	F	21	4.65
144025	MC	1982	ad	F	21	5.72
144035	MC	1982	ad	F	22	5.85
144033	MC	1982	ad	M	23	6.44
144022	MC	1982	ad	M	23	6.51
144020	MC	1982	ad	M	21	6.55

144026	MC	1982	ad	M	26	6.68
144037	MC	1982	ad	F	22	6.87
144023	MC	1982	ad	F	24	6.91
144027	MC	1982	ad	M		7.09
144017	MC	1982	ad	M	23	7.30
144029	MC	1982	ad	F	20	7.33
146943	MC	1982	imm	M	25.5	7.38
146933	MC	1982	imm	F	24	7.46
144031	MC	1982	ad	F	25	7.78
144021	MC	1982	ad	M	22	7.80
144036	MC	1982	ad	F	21	7.88
144019	MC	1982	ad	F	22	7.99
146927	MC	1982	imm	M	23	8.02
146940	MC	1982	ad	M	25.5	8.17
144034	MC	1982	ad	F	23	8.23
146938	MC	1982	ad	M	20	8.24
146931	MC	1982	ad	F	23	8.39
146926	MC	1982	ad	F	25	8.65
144028	MC	1982	ad	M	23	8.72
146935	MC	1982	imm	F	24	8.84
146934	MC	1982	ad	F	23	8.91
146941	MC	1982	imm	F	25	8.95
146942	MC	1982	ad	F	22	9.23
146928	MC	1982	ad	M	24.8	9.27
146929	MC	1982	ad	M	25.5	9.44
146925	MC	1982	imm	M	25	9.47
146924	MC	1982	imm	M	25	9.50
146939	MC	1982	ad	F	24.5	9.71
146932	MC	1982	ad	F	20.5	10.39
146936	MC	1982	ad	F	23.5	10.74
148045	MC	1983	ad	F	25	4.01
148039	MC	1983	ad	F	24	6.64
148034	MC	1983	ad	M	26.2	7.73
148028	SC	1983	ad	M	26	8.03
148024	SC	1983	imm	F	23.9	8.07
148030	SC	1983	imm	M	23.5	8.08
148026	SC	1983	ad	M	23.9	8.24
148040	MC	1983	imm	M	25.5	8.37
148036	MC	1983	imm	F	23.5	8.85
148044	MC	1983	ad	F	23.5	8.86
148051	MC	1983	ad	F	23	8.97
148033	MC	1983	imm	M	23	9.00
148025	SC	1983	ad	F	27	9.20
148020	SC	1983	imm	M	24	9.24
148043	MC	1983	imm	M	23.5	9.32

148023	SC	1983	imm	F	24	9.48
148038	MC	1983	imm	F	22.2	9.71
148022	SC	1983	imm	M	25.1	9.72
148037	MC	1983	imm	F	21.9	9.77
148046	MC	1983	imm	M	23.5	9.83
148041	MC	1983	ad	M	24.5	10.13
148016	SC	1983	ad	F	26.8	10.26
148050	MC	1983	ad	F	25.5	10.28
148021	SC	1983	ad	M	25.1	10.32
148049	MC	1983	ad	M	26	10.47
148018	SC	1983	ad	F	24.5	10.58
148027	SC	1983	ad	F	23.5	10.61
148032	MC	1983	ad	F	24	10.71
148042	MC	1983	imm	F	24.5	10.85
148017	SC	1983	ad	F	24.1	10.93
148015	SC	1983	imm	F	22	11.14
148053	MC	1983	ad	M	26.3	11.34
148031	SC	1983	imm	F	22.5	11.34
148052	MC	1983	ad	M	25	11.35
148019	SC	1983	imm	F	24.2	11.78
148047	MC	1983	imm	F	23.5	12.19
148055	MC	1983	ad	F	24	13.20
148029	SC	1983	ad	M	27.3	13.98
148048	MC	1983	imm	M	26.2	14.96
148054	MC	1983	imm	M	23	16.21
151427	GC	1985	imm	M	22	3.95
151436	GC	1985	imm	F	21.5	4.38
151445	GC	1985	ad	F	23.5	4.47
151437	GC	1985	ad	M	23	4.92
151448	GC	1985	imm	F	21	4.97
151442	GC	1985	imm	M	20.5	5.26
151444	GC	1985	imm	M	26	5.30
151435	GC	1985	imm	F	24.5	5.44
151443	GC	1985	imm	M	23	5.48
151441	GC	1985	imm	F	21	5.51
151426	GC	1985	imm	M	23.2	5.55
151438	GC	1985	ad	M	25.5	5.60
151446	GC	1985	ad	F	24	5.63
151439	GC	1985	imm	F	22.5	5.65
151440	GC	1985	imm	M	24.5	5.80
150238	GC	1985	ad	M	23	7.28
150264	GC	1985	ad	M	24.4	8.63
150235	GC	1985	ad	F	19.6	8.76
150245	GC	1985	ad	F	24.7	9.42
150253	GC	1985	ad	M	23.5	9.67

151424	GC	1985	imm	F	25	9.69
150270	GC	1985	imm	M	24.9	9.78
150268	GC	1985	ad	M	22.6	9.84
150255	GC	1985	ad	M	23.9	10.01
150226	GC	1985	imm	F	24.9	10.03
150234	GC	1985	ad	M	24.1	10.03
150265	GC	1985	ad	M	22.5	10.15
150269	GC	1985	ad	M	24.4	10.22
150252	GC	1985	ad	M	22.5	10.27
151415	MC	1985	ad	F	24.3	10.40
151408	MC	1985	ad	F	21.5	10.46
151412	MC	1985	imm	M	23	10.46
150215	GC	1985	imm	M	23.5	10.52
150223	GC	1985	ad	F	24.3	10.82
151417	MC	1985	imm	F	24	11.04
150221	GC	1985	ad	M	24.6	11.07
151409	MC	1985	ad	M	21	11.13
150251	GC	1985	imm	F	22.2	11.25
150263	GC	1985	ad	F	22.8	11.25
150250	GC	1985	imm	M	23.3	11.30
150228	GC	1985	imm	F	22.4	11.37
150257	GC	1985	imm	F	22	11.38
151413	MC	1985	imm	F	22	11.50
150258	GC	1985	ad	F	21.4	11.60
150225	GC	1985	ad	F	22.5	11.61
150243	GC	1985	ad	M	25	11.71
151416	MC	1985	imm	M	24.5	11.72
150239	GC	1985	ad	F	24.4	11.75
150247	GC	1985	ad	M	24.4	11.83
150248	GC	1985	ad	F	21.1	11.93
150256	GC	1985	imm	F	21.1	11.98
150260	GC	1985	imm	F	23	12.19
150220	GC	1985	ad	M	25.3	12.23
150246	GC	1985	ad	M	22.6	12.33
151425	GC	1985	imm	M	26	12.40
150222	GC	1985	ad	F	21.2	12.49
150267	GC	1985	imm	F	23.6	12.50
150230	GC	1985	ad	M	22.4	12.57
151418	MC	1985	imm	M	22.5	12.61
150262	GC	1985	ad	F	23.6	12.75
151410	MC	1985	ad	F	21.5	12.77
151407	MC	1985	imm	M	21	12.84
150249	GC	1985	ad	F	23.2	12.88
150241	GC	1985	ad	M	23	13.07
150242	GC	1985	ad	M	23.5	13.18

150224	GC	1985	ad	M	23	13.40
150244	GC	1985	imm	F	24.1	13.43
151422	GC	1985	ad	F	21.5	13.78
150216	GC	1985	ad	F	22	13.85
151421	GC	1985	imm	M	24.8	13.90
150266	GC	1985	imm	M	21.9	14.01
150229	GC	1985	ad	M	23.1	14.21
150261	GC	1985	ad	F	22.5	14.22
150219	GC	1985	ad	M	23.9	14.64
150227	GC	1985	ad	M	22.6	15.11
150240	GC	1985	ad	F	23	15.21
151419	MC	1985	ad	M	23	15.38
150232	GC	1985	ad	F	22.5	15.91
151414	MC	1985	ad	F	23.5	16.20
151423	GC	1985	ad	F	24	16.70
150259	GC	1985	imm	M	23.4	17.35
150254	GC	1985	imm	M	23.4	18.24
150237	GC	1985	ad	M	23	18.49
151518	MC	1986	ad	M	22.5	4.58
151592	MC	1986	ad	F	21.9	4.68
152616	MC	1986	ad	F	20	4.89
152607	MC	1986	ad	M	20.5	4.98
151513	MC	1986	ad	F	22.5	5.12
151524	MC	1986	ad	M	23	5.12
152623	MC	1986	imm	F	20.5	5.13
151519	MC	1986	ad	M	22	5.19
151602	MC	1986	ad	M	22.4	5.19
152312	GC	1986	imm	M	20.6	5.24
151514	MC	1986	ad	M	20.5	5.30
151528	MC	1986	ad	M	22	5.31
151599	MC	1986	ad	M	24	5.32
152592	MC	1986	ad	M	23.5	5.47
152612	MC	1986	ad	F	21.2	5.57
151596	MC	1986	ad	M	23.4	5.59
151604	MC	1986	ad	F	21.7	5.61
151607	MC	1986	ad	M	24.3	5.63
152608	MC	1986	ad	F	19.5	5.63
151520	MC	1986	ad	M	22	5.67
151593	MC	1986	ad	F	25.4	5.72
151589	MC	1986	ad	M	25	5.75
151595	MC	1986	imm	F	21.2	5.83
151521	MC	1986	ad	F	22	5.83
151522	MC	1986	ad	M	22.5	5.83
152620	MC	1986	imm	F	21.8	5.83
151515	MC	1986	ad	M	23	5.88

151597	MC	1986	ad	M	22.4	5.96
151525	MC	1986	ad	M	21	5.98
152313	GC	1986	imm	M	20.5	6.04
151516	MC	1986	ad	F	21	6.06
151594	MC	1986	ad	F	22.2	6.08
152615	MC	1986	imm	M	22.5	6.23
151523	MC	1986	ad	M	22	6.28
152297	GC	1986	imm	M	20	6.31
152594	MC	1986	ad	F	21.3	6.33
152614	MC	1986	ad	M	21	6.35
152595	MC	1986	ad	M	21	6.36
151588	MC	1986	ad	F	24	6.42
151586	MC	1986	ad	F	25.5	6.42
151590	MC	1986	ad	M	25	6.45
151587	MC	1986	imm	M	23	6.48
152309	GC	1986	imm	M	19.4	6.59
151606	MC	1986	ad	F	23.7	6.63
152609	MC	1986	imm	M	20.7	6.65
152611	MC	1986	imm	M	19.8	6.66
152618	MC	1986	imm	F	22.5	6.81
151598	MC	1986	ad	M	21.8	6.86
151601	MC	1986	ad	M	22.1	6.89
152299	MC	1986	imm	F	19.8	6.96
151600	MC	1986	ad	F	21.1	7.07
152617	MC	1986	ad	F	22.8	7.31
152610	MC	1986	imm	M	19.3	7.36
151529	MC	1986	ad	F	22	7.37
152593	MC	1986	ad	F	22.5	7.43
152624	MC	1986	ad	F	22	7.47
152318	GC	1986	imm	M	21.5	7.68
152303	MC	1986	imm	M	20	7.76
151517	MC	1986	ad	F	21	7.82
152626	MC	1986	imm	F	22.5	7.94
151591	MC	1986	ad	M	21.4	8.05
152625	MC	1986	ad	M	22	8.08
152621	MC	1986	ad	F	20.5	8.13
152622	MC	1986	imm	M	22.5	8.36
151603	MC	1986	ad	F	21.5	8.40
152606	MC	1986	ad	M	19.8	8.47
152619	MC	1986	ad	M	21.5	8.85
156090	GC	1990	ad	M	19.7	6.13
156083	GC	1990	imm	M	23.9	6.44
156096	GC	1990	ad	F	22	6.60
156095	GC	1990	imm	F	19.5	6.69
156098	GC	1990	ad	F	20.5	7.00

156092	GC	1990	imm	M	21	7.30
156091	GC	1990	ad	M	19.5	7.61
156085	GC	1990	ad	F	20	9.92

Appendix 2: Linear Regressions of Feather CORT and Spatial or Weather Data

Table A2.1: Results of simple linear regressions exploring one-way relationships between feather CORT of house sparrows sampled from 49 sites across Mexico from Dec. 2006 – March 2007 and various spatial and weather variables. Significant p-values (α=0.05) are bolded.

X variable	Residual SE	F-statistic	df	R-squared	p-value
Latitude	2.509	19.89	446	0.0427	**1.04×10^{-5}**
Longitude	2.563	0.7311	446	0.001636	0.393
Elevation	2.558	2.374	446	0.005295	0.124
January precipitation	2.549	5.64	446	0.01249	**0.018**
February precipitation	2.562	0.9736	446	0.002178	0.324
March precipitation	2.545	6.899	446	0.01523	**0.00892**
April precipitation	2.56	1.496	446	0.003342	0.222
May precipitation	2.557	2.827	446	0.006299	0.0934
June precipitation	2.543	7.82	446	0.01723	**0.00539**
July precipitation	2.555	3.264	446	0.007266	0.0715
August precipitation	2.545	6.935	446	0.01531	**0.00875**
September precipitation	2.553	4.019	446	0.00893	**0.0456**
October precipitation	2.537	9.735	446	0.02136	**0.00192**
November precipitation	2.534	10.76	446	0.02355	**0.00112**
December precipitation	2.527	13.42	446	0.02921	**0.000279**
Mean annual precipitation	2.542	7.902	446	0.01741	**0.00515**
January minimum temperature	2.496	24.86	446	0.0528	**8.83×10^{-7}**
February minimum temperature	2.498	24.01	446	0.05109	**1.34×10^{-6}**
March minimum temperature	2.507	20.65	446	0.04425	**7.11×10^{-6}**
April minimum temperature	2.543	7.571	446	0.01669	**0.00617**
May minimum temperature	2.555	3.445	446	0.007666	0.0641
June minimum temperature	2.562	0.9462	446	0.002117	0.331
July minimum temperature	2.564	0.3257	446	0.0007298	0.568
August minimum temperature	2.562	0.934	446	0.00209	0.334
September minimum temperature	2.552	4.591	446	0.01019	**0.0327**
October minimum temperature	2.531	12.02	446	0.02625	**0.000577**
November minimum temperature	2.511	19.39	446	0.04167	**1.33×10^{-5}**
December minimum temperature	2.497	24.5	446	0.05206	**1.06×10^{-6}**
January maximum temperature	2.466	36.46	446	0.07557	**3.29×10^{-9}**
February maximum temperature	2.463	37.58	446	0.07772	**1.93×10^{-9}**
March maximum temperature	2.482	30.14	446	0.06331	**6.74×10^{-8}**
April maximum temperature	2.534	10.73	446	0.02348	**0.00114**
May maximum temperature	2.564	0.1273	446	0.0002852	0.721
June maximum temperature	2.554	3.707	446	0.008244	0.0548
July maximum temperature	2.562	0.8369	446	0.001873	0.361
August maximum temperature	2.565	0.05264	446	0.000118	0.819
September maximum temperature	2.563	0.4317	446	0.0009671	0.511
October maximum temperature	2.549	5.552	446	0.0123	**0.0189**

November maximum temperature	2.499	23.84	446	0.05073	**1.46×10^{-6}**
December maximum temperature	2.474	33.43	446	0.06973	**1.39×10^{-8}**
January *d*-excess	2.56	1.534	446	0.003429	0.216
February *d*-excess	2.558	2.498	446	0.005569	0.115
March *d*-excess	2.531	12.01	446	0.02623	**0.00058**
April *d*-excess	2.502	22.74	446	0.04852	**2.51×10^{-6}**
May *d*-excess	2.554	3.801	446	0.008451	0.0518
June *d*-excess	2.564	0.08338	446	0.0001869	0.773
July *d*-excess	2.562	0.8312	446	0.00186	0.362
August *d*-excess	2.552	4.501	446	0.009992	**0.0344**
September *d*-excess	2.565	2.324×10^{-6}	446	5.211×10^{-9}	0.999
October *d*-excess	2.547	6.306	446	0.01394	**0.0124**
November *d*-excess	2.565	0.03591	446	8.051×10^{-5}	0.85
December *d*-excess	2.533	11.23	446	0.02456	**0.000873**
Mean annual *d*-excess	2.564	0.169	446	0.0003787	0.681

Table A2.2: Results of simple linear regressions exploring one-way relationships between feather CORT of Eurasian tree sparrows sampled from 4 counties in Illinois from 1963-1990 and monthly weather and drought variables from the pre-moult (April-June) and moult (July-October) periods. Significant p-values (α=0.05) are bolded.

X variable	**Resid. SE**	**F-stat**	**df**	**R^2**	**p-value**
number of days in the April with ≥0.1 inch precipitation	2.71	25.95	431	0.05679	**5.24×10^{-7}**
number of days in the May with ≥0.1 inch precipitation	2.788	0.6116	431	0.001417	0.435
number of days in the June with ≥0.1 inch precipitation	2.768	6.945	431	0.01586	**0.00871**
number of days in the July with ≥0.1 inch precipitation	2.79	0.2486	431	0.0005764	0.618
number of days in the August with ≥0.1 inch precipitation	2.748	13.56	431	0.0305	*0.000261*
number of days in the September with ≥0.1 inch precipitation	2.776	4.543	431	0.01043	*0.0336*
number of days in the October with ≥0.1 inch precipitation	2.582	72.23	431	0.1435	**3.16×10^{-16}**
number of days in the April with ≥0.5 inch precipitation	2.604	64.1	431	0.1295	**1.11×10^{-14}**
number of days in the May with ≥0.5 inch precipitation	2.638	51.19	431	0.1062	**3.63×10^{-12}**
number of days in the June with ≥0.5 inch precipitation	2.771	5.937	431	0.01359	**0.0152**
number of days in the July with ≥0.5	2.73	19.35	431	0.04297	**1.37×10^{-5}**

inch precipitation					
number of days in the August with ≥0.5 inch precipitation	2.772	5.621	431	0.01287	**0.0182**
number of days in the September with ≥0.5 inch precipitation	2.789	0.3469	431	0.0008042	0.556
number of days in the October with ≥0.5 inch precipitation	2.756	10.82	431	0.02449	**0.00109**
number of days in the April with ≥1.0 inch precipitation	2.689	33.2	431	0.07152	**1.58×10^{-8}**
number of days in the May with ≥1.0 inch precipitation	2.633	53.03	431	0.1096	**1.57×10^{-12}**
number of days in the June with ≥1.0 inch precipitation	2.782	2.523	431	0.00582	0.113
number of days in the July with ≥1.0 inch precipitation	2.785	1.654	431	0.003823	0.199
number of days in the August with ≥1.0 inch precipitation	2.641	50.1	431	0.1041	**5.97×10^{-12}**
number of days in the September with ≥1.0 inch precipitation	2.757	10.63	431	0.02407	**0.0012**
number of days in the October with ≥1.0 inch precipitation	2.769	6.747	431	0.01541	**0.00971**
April departure from normal monthly precipitation	2.718	23.37	431	0.05143	**1.86×10^{-6}**
May departure from normal monthly precipitation	2.703	28.36	431	0.06174	**1.62×10^{-7}**
June departure from normal monthly precipitation	2.787	1.099	431	0.002544	0.295
July departure from normal monthly precipitation	2.749	12.98	431	0.02924	**0.000351**
August departure from normal monthly precipitation	2.648	47.5	431	0.09926	**1.97×10^{-11}**
September departure from normal monthly precipitation	2.79	0.06183	431	0.0001434	0.804
October departure from normal monthly precipitation	2.712	25.36	431	0.05557	**7.01×10^{-7}**
April departure from normal monthly temperature	2.611	61.21	431	0.1243	**4.01×10^{-14}**
May departure from normal monthly temperature	2.675	38.13	431	0.08128	**1.53×10^{-9}**
June departure from normal monthly temperature	2.713	24.86	431	0.05453	**8.97×10^{-7}**
July departure from normal monthly temperature	2.765	8.088	431	0.01842	**0.00467**
August departure from normal monthly temperature	2.587	70.3	431	0.1402	**7.3×10^{-16}**
September departure from normal	2.746	14.19	431	0.03187	**0.000188**

monthly temperature					
October departure from normal monthly temperature	2.728	19.91	431	0.04416	**1.04×10^{-5}**
number of days in April with snow ≥1 inch	2.754	11.55	431	0.02609	**0.000742**
number of days in April with minimum temperature ≤32 ºF	2.79	0.02173	431	5.04×10^{-5}	0.883
number of days in May with minimum temperature ≤32 ºF	2.784	1.902	431	0.004395	0.169
number of days in September with minimum temperature ≤32 ºF	2.635	52.52	431	0.1086	**1.98×10^{-12}**
number of days in October with minimum temperature ≤32 ºF	2.701	28.88	431	0.06279	**1.27×10^{-7}**
number of days in April with maximum temperature ≥90 ºF	2.777	4.271	431	0.009813	**0.0394**
number of days in May with maximum temperature ≥90 ºF	2.665	41.66	431	0.08813	**2.93×10^{-10}**
number of days in June with maximum temperature ≥90 ºF	2.787	1.151	431	0.002662	0.284
number of days in July with maximum temperature ≥90 ºF	2.784	1.935	431	0.004469	0.165
number of days in August with maximum temperature ≥90 ºF	2.678	36.9	431	0.07885	**2.74×10^{-9}**
number of days in September with maximum temperature ≥90 ºF	2.787	1.125	431	0.002603	0.289
number of days in October with maximum temperature ≥90 ºF	2.753	11.96	431	0.027	**0.000598**
April extreme minimum temperature	2.779	3.454	431	0.007949	0.0638
May extreme minimum temperature	2.75	12.87	431	0.029	**0.000372**
June extreme minimum temperature	2.785	1.546	431	0.003574	0.214
July extreme minimum temperature	2.74	15.88	431	0.03554	**7.92×10^{-5}**
August extreme minimum temperature	2.741	15.81	431	0.03537	**8.23×10^{-5}**
September extreme minimum temperature	2.418	142.9	431	0.249	**$<2 \times 10^{-16}$**
October extreme minimum temperature	2.763	8.633	431	0.01964	**0.00348**
April extreme daily maximum precipitation	2.785	1.617	431	0.003737	0.204
May extreme daily maximum precipitation	2.773	5.55	431	0.01271	**0.0189**
June extreme daily maximum precipitation	2.758	10.32	431	0.02338	**0.00142**
July extreme daily maximum precipitation	2.78	3.264	431	0.007517	0.0715
August extreme daily maximum precipitation	2.528	94.28	431	0.1795	**$<2 \times 10^{-16}$**

September extreme daily maximum precipitation	2.775	4.805	431	0.01103	**0.0289**
October extreme daily maximum precipitation	2.787	1.027	431	0.002376	0.312
April extreme maximum temperature	2.789	0.4909	431	0.001138	0.4839
May extreme maximum temperature	2.564	79.47	431	0.1557	**$<2 \times 10^{-16}$**
June extreme maximum temperature	2.725	20.79	431	0.04603	**6.67×10^{-6}**
July extreme maximum temperature	2.78	3.12	431	0.007187	0.0781
August extreme maximum temperature	2.701	29.11	431	0.06326	**1.13×10^{-7}**
September extreme maximum temperature	2.529	93.72	431	0.1786	**$<2 \times 10^{-16}$**
October extreme maximum temperature	2.784	2.144	431	0.00495	0.144
April heating degree days	2.567	78.45	431	0.154	**$<2 \times 10^{-16}$**
May heating degree days	2.69	32.76	431	0.07064	**1.95×10^{-8}**
June heating degree days	2.788	0.6168	431	0.001429	0.433
July heating degree days	2.779	3.584	431	0.008247	0.059
August heating degree days	2.72	22.58	431	0.04978	**2.75×10^{-6}**
September heating degree days	2.446	129.9	431	0.2316	**$<2 \times 10^{-16}$**
October heating degree days	2.736	17.17	431	0.03832	**4.11×10^{-5}**
April mean minimum temperature	2.667	40.68	431	0.08624	**4.64×10^{-10}**
May mean minimum temperature	2.648	47.69	431	0.09963	**1.8×10^{-11}**
June mean minimum temperature	2.753	11.93	431	0.02694	**0.000607**
July mean minimum temperature	2.707	26.96	431	0.05888	**3.20×10^{-7}**
August mean minimum temperature	2.753	11.87	431	0.0268	**0.000627**
September mean minimum temperature	2.669	39.97	431	0.08487	**6.44×10^{-10}**
October mean minimum temperature	2.694	31.28	431	0.06767	**3.97×10^{-8}**
April mean maximum temperature	2.558	81.97	431	0.1598	**$<2 \times 10^{-16}$**
May mean maximum temperature	2.67	39.82	431	0.08458	**6.91×10^{-10}**
June mean maximum temperature	2.779	3.488	431	0.008028	0.0625
July mean maximum temperature	2.755	11.16	431	0.02523	**0.00091**
August mean maximum temperature	2.644	48.89	431	0.1019	**1.04×10^{-11}**
September mean maximum temperature	2.739	16.48	431	0.03682	**5.85×10^{-5}**
October mean maximum temperature	2.788	0.6626	431	0.001535	0.4161
April mean temperature	2.579	73.75	431	0.1461	**$<2 \times 10^{-16}$**
May mean temperature	2.651	46.69	431	0.09774	**2.85×10^{-11}**
June mean temperature	2.765	7.92	431	0.01804	**0.00511**
July mean temperature	2.724	21.36	431	0.04722	**5.04×10^{-6}**
August mean temperature	2.688	33.64	431	0.0724	**1.29×10^{-8}**
September mean temperature	2.678	37.08	431	0.07923	**2.51×10^{-9}**
October mean temperature	2.765	8.031	431	0.01829	**0.00481**
April maximum snow depth	2.778	3.893	431	0.008952	**0.0491**
April total precipitation	2.692	31.96	431	0.06904	**2.86×10^{-8}**
May total precipitation	2.693	31.72	431	0.06855	**3.22×10^{-8}**
June total precipitation	2.79	0.2433	431	0.0005642	0.622
July total precipitation	2.771	6.08	431	0.01391	**0.0141**

August total precipitation	2.69	32.93	431	0.07098	**1.8×10^{-8}**
September total precipitation	2.787	0.9609	431	0.002224	0.328
October total precipitation	2.715	24.21	431	0.05318	**1.23×10^{-6}**
April total snowfall	2.785	1.83	431	0.004228	0.177
April Palmer Drought Severity Index	2.513	100.4	431	0.1889	**$< 2 \times 10^{-16}$**
May Palmer Drought Severity Index	2.494	108.5	431	0.2011	**$< 2 \times 10^{-16}$**
June Palmer Drought Severity Index	2.789	0.5127	431	0.001188	0.474
July Palmer Drought Severity Index	2.779	3.426	431	0.007887	0.0649
August Palmer Drought Severity Index	2.729	19.69	431	0.04369	**1.16×10^{-5}**
September Palmer Drought Severity Index	2.79	5.3×10^{-6}	431	1.23×10^{-8}	0.998
October Palmer Drought Severity Index	2.741	15.56	431	0.03485	**9.32×10^{-5}**

Appendix 3: Radioimmunoassay Data

Table A3.1: Radioimmunoassay parameters for the 10 assays used to measure CORT levels in feathers of house sparrows from Mexico. Total count ('TC') values should be close to 5000 CPM. ED20, ED50, and ED80 are CORT levels in pg/100 μL at 3 points on the standard curve: 20% bound, 50% bound, and 80% bound.

Assay #	TC	% Binding	% NSB	ED20	ED50	ED80	CV (%)
1	4625.5	28.06	4.12	178.31	45.69	11.71	6.28
2	4730.5	26.45	5.63	156.56	40.71	8.11	7.33
3	4619.5	27.47	4.99	175.04	43.57	10.83	4.50
4	4623	28.28	4.23	155.25	40.96	10.89	6.98
5	4548.5	28.20	4.24	159.94	41.12	9.89	8.15
6	4320	28.92	3.97	173.04	39.60	8.82	5.41
7	4675	29.19	5.63	144.72	42.50	10.45	10.60
8	4631	28.58	4.33	169.24	41.46	10.01	5.01
9	4094	28.08	3.59	159.90	40.37	10.18	6.36
10	4804	28.13	4.12	187.68	41.52	9.18	5.04

Table A3.2: Radioimmunoassay parameters for the 9 assays used to measure CORT levels in feathers of Eurasian tree sparrows from Illinois. Total count ('TC') values should be close to 5000 CPM. ED20, ED50, and ED80 are CORT levels in pg/100 μL at 3 points on the standard curve: 20% bound, 50% bound, and 80% bound.

Assay #	TC	% Binding	% NSB	ED20	ED50	ED80	CV (%)
1	4078.5	28.85	3.58	183.37	47.70	12.77	5.90
2	4215.5	27.49	5.11	167.07	45.00	11.38	5.82
3	4291.5	26.86	3.70	179.08	43.04	10.34	6.68
4	4674	28.06	4.60	165.97	42.23	10.64	5.99
5	4683.5	29.01	3.83	170.30	43.73	10.90	4.84
6	4676	30.14	3.96	146.90	37.63	8.19	4.00
7	4747	27.63	3.22	202.66	42.46	8.75	3.90
8	4625.5	26.86	3.92	163.09	41.50	10.03	6.00
9	4804.5	28.13	4.12	187.68	41.52	9.18	5.04